Spider Diversity and Ecology in Native Tussock Grasslands of the South Island, New Zealand

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by
J. Malumbres Olarte

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Tussock grasslands have been the subject of relatively extensive botanical research as they are considered ecologically distinctive and economically important. However, comparatively little is known about the invertebrate fauna of tussock grasslands, and more particularly, almost nothing is known about the spider fauna. The aim of this thesis is to evaluate methods for studying spiders in tussock grasslands, and to answer some questions about their ecological interactions with vegetation and the effects of fire on tussock grassland spider communities.

The selection of appropriate sampling methods is crucial for the success of ecological studies. An evaluation of spider sampling methods in tussock grassland identifies pitfall traps as the most effective and efficient. The physical characteristics of tussocks limit the efficiency of some sampling techniques, such as suction sampling. Methods that target the lowest layers of vegetation, such as pitfall traps, should be used for the study of spider community in tussock grasslands, although other methods, such as foliage beating, may also be considered to collect unique species.

Molecular methods and analyses have the potential to add value to ecological data and help answer ecological questions. Mitochondrial DNA analyses are used for gender matching, and discrimination and identification of undescribed species. The results of this study show that analytical methods, such as GMYC, developed for DNA identification or taxonomy, may not always be congruent with morphological information and may require data from other sources. Nevertheless, the use of DNA data should be considered in ecological

studies as they can provide crucial supplementary information for specimen identification, preventing incorrect conclusions.

Little is known about the factors that drive spider diversity in tussock grasslands. Environmental factors, such as soil moisture, affect vegetation structure in tussock ecosystems, which in turn determine spider assemblages. Gradients in vegetation are matched by spider assemblages, with the family Orsolobidae favouring areas with marshland vegetation, and aerial-web builders, such as Linyphiidae, preferring shrubby vegetation. Species of the genus *Anoteropsis* (Lycosidae), identified as potential indicators of the structure of the vegetation, are recommended to be included in monitoring programs for conservation management.

The effects of fire on spider communities are assessed through a long-term experiment with burnt and unburnt plots spanning years before and after a fire. Spider diversity decreases drastically after the fire and remains low for four years. Although the overall trend is a decrease in the abundance of most spider families, Linyphiidae shows a large increase in the years following the fire, which is explained by their ability to disperse and colonise new habitats. An increase in the number of exotic species, particularly *Diplocephalus cristatus*, is behind this trend, showing the importance of addressing the question of the effects of disturbances on native and exotic species in New Zealand ecosystems, and the dangers that they pose to native biodiversity.

This thesis contributes significantly to the understanding of spider communities in one of the most important native ecosystems in New Zealand, providing fundamental methodological information for future studies and unveiling some of the key drivers of spider diversity.

Keywords— spiders, tussock grasslands, sampling methods, DNA identification, DNA taxonomy, diversity patterns, habitat requirements, vegetation structure, community structure, fire, exotic species, conservation management.

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Abbreviations

ACE: Abundance-based coverage estimator of species richness

AIC: Akaike information criterion

amsl: Above mean sea level

ANOSIM: Analysis of similarities

ANOVA: Analysis of variance

BIC: Bayesian information criterion

COI: Cytochrome *c* oxidase subunit 1

dNTP: Deoxyribonucleotide triphosphate

DNA: Deoxyribonucleic acid

Γ: Gamma parameter in phylogenetic models

GMYC: General mixed Yule-coalescent model

GTR: General time reversible (phylogenetic) model

ICE: Incidence-based coverage estimator of species richness

IndVal: Indicator value

K2P: Kimura-2-Parameter

MCMC: Markov chain Monte Carlo method

ML: Maximum likelihood analysis

PCA: Principal component analysis

PCR: Polymerase chain reaction

S_{17}: Steinhaus dissimilarity coefficient

S_8: Sørensen dissimilarity coefficient

TIM1: Transitional (phylogenetic) model 1

TIM3: Transitional (phylogenetic) model 3

TrN: Phylogenetic model developed by Tamura and Nei

Chapter 1

Introduction

1.1 Background

1.1.1 The importance of studying arthropods

Invertebrates, and more particularly arthropods, are a major part of biodiversity (Wilson, 1987). Although over a million species of arthropods have been described so far (Stork, 1988), this number probably only represents a fraction of their total global diversity. The omnipresence of arthropods in almost every ecosystem on earth and their impressive ability to exploit resources make them an ideal source of information for conservation management (Bell et al., 2001; Gering et al., 2003; McGuinness, 2001; Schmidt et al., 2008) and the subject of monitoring programs (Cristofoli et al., 2010; Doran et al., 1999; Hopp et al., 2010).

1.1.2 Spiders within a system

Among arthropods, spiders play a particularly important role in ecological processes. Spiders are known for their great species richness and diversity of predatory behaviours (Coddington et al., 1996; Coddington and Levi, 1991), with around 41,000 described species (Platnick, 2010) out of an estimated 500,000 species worldwide (Grove and Stork, 2000). Their impact as generalist predators on invertebrate herbivores (Birkhofer et al., 2007; Clarke and Grant, 1968; Wise, 2006) is an indicator of their key role in the trophic webs of most terrestrial ecosystems (Nyffeler, 2000; Wise, 1993). Spiders respond to changing habitat conditions (Uetz, 1991; Ziesche and Roth, 2008) either di-

rectly, or through changes in the physical characteristics of their environment (Dennis, 2003; Dennis et al., 2001; Greenstone, 1984) and in the populations of their prey (Marc et al., 1999; Schmitz, 2003). Hence, they are considered appropriate organisms for the study of succession processes or modifications to ecosystems caused by human or natural disturbances (Buddle et al., 2000; Maelfait and Hendrickx, 1998; Marc et al., 1999) and for their overall potential as ecological indicators (Churchill, 1997).

1.1.3 Lack of knowledge on New Zealand spiders

Despite the extensive taxonomic work carried out by arachnologists like the late Ray Forster (1922-2000) over 50 years, little ecological research has been carried out on New Zealand spiders. There have been few examples of ecological studies that have focused on the role of spiders in agroecosystems (Clark et al., 2004; McLachlan and Wratten, 2003; Sivasubramaniam et al., 1997; Topping and Lövei, 1997; Vink et al., 2004). However, spider communities in native ecosystems have drawn limited attention (Alley et al., 2001; Derraik et al., 2005; Moeed and Meads, 1985, 1986; Topping and Lövei, 1997). Tussock grasslands have been no exception and only general trends in spider communities have been covered in a series of studies (Barratt et al., 2009, 2005; Topping and Lövei, 1997).

1.1.4 Tussock grasslands of New Zealand

The area covered by grasslands indigenous to New Zealand has been reduced dramatically over the last 150 years, mainly due to their exploitation and transformation into pasture for livestock (Mark et al., 2005) by European settlers (Figure 1.1). Despite this, indigenous grasslands still cover approximately 40% of the land cover of New Zealand (Barratt et al., 2005; Wardle, 1991) and their economic and ecological values have been increasingly recognised over the last few decades (Brockerhoff et al., 2008; Mark et al., 2009). Efforts to protect, conserve and restore them have resulted in the creation of tussock conservation areas, such as Te Papanui Conservation Park in Central Otago. Research has been conducted on the botanical component of tussock grasslands (Barker, 1953; Mark, 1969; Mark et al., 2009; Mark, 1993). Unfor-

tunately, the invertebrate fauna of these ecosystems has been the subject of very few studies.

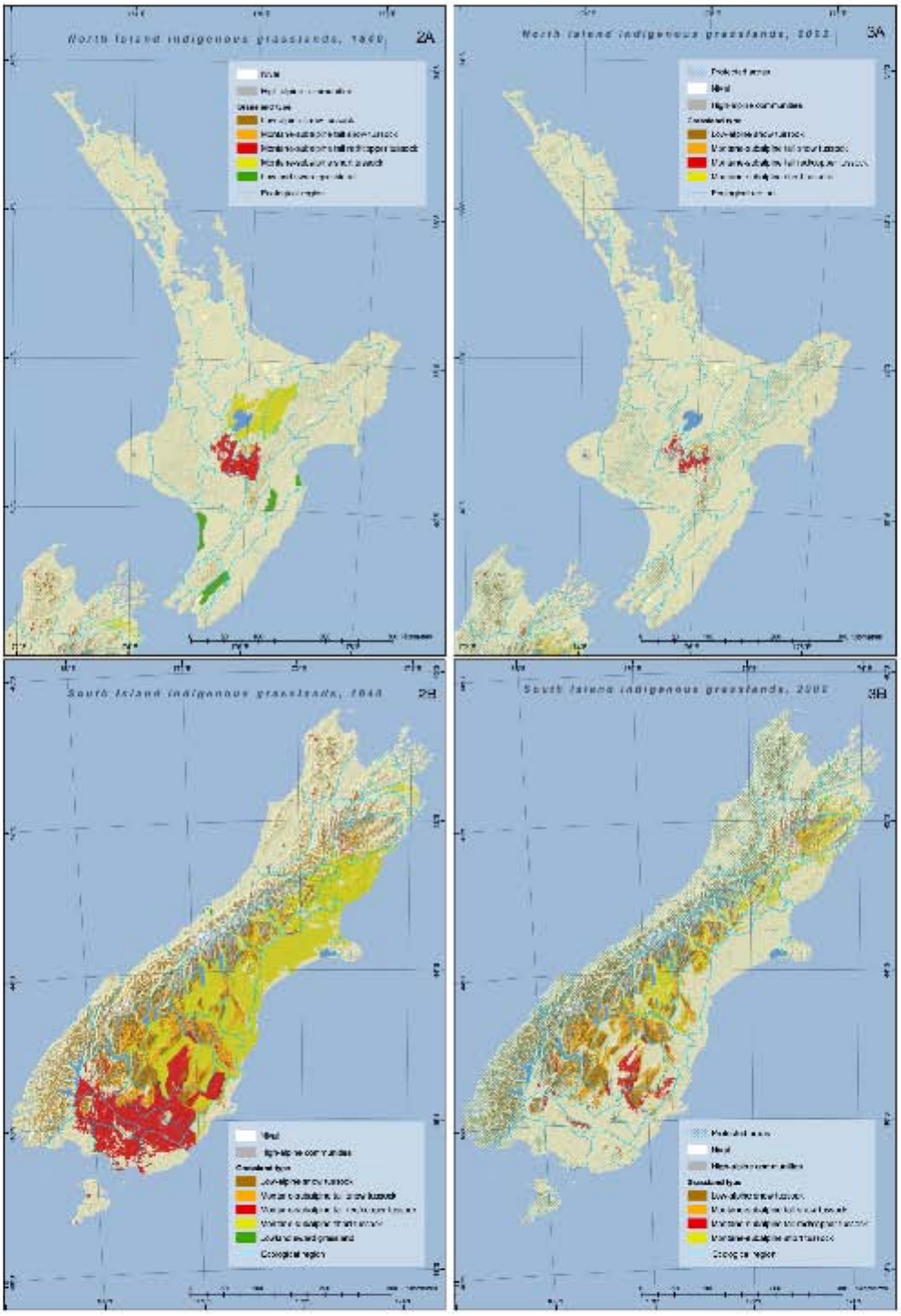

Figure 1.1: Distribution and extent of the main types of indigenous grasslands of New Zealand in the North (2A and 3A) and South (2B and 3B) Islands in the early 1840s and 2002 (figures from Mark et al., 2005). Maps of the distributions in the 1840s are estimated.

This thesis aims to be the first - and hopefully not the last - set of ecolog-

ical studies looking at natural and human factors that drive spider diversity in New Zealand tussock grasslands, from guild composition to species level. More specifically, this thesis investigates the response of spider assemblages to variation in plant structure and composition and to fire as a common human disturbance in tussock grasslands. In addition, the efficiency of different sampling methods in tussock grasslands and the potential of molecular tools for completion of ecological data are evaluated.

1.2 Structure of the thesis

The thesis is divided into an introduction (**chapter one**), four chapters written as separate manuscripts without references, a summation chapter (**chapter six**), and general references and appendices. As the four middle chapters will be submitted for publication, certain information may be repeated, especially in the introductory sections, although this has been minimised where possible. There are three overall goals in this thesis.

1. Characterise the arachnofauna of native tussock grasslands of New Zealand.

2. Provide information necessary for the improvement and standardisation of methods for spider collection and identification.

3. Identify the environmental and human factors that influence spider assemblages in tussock grasslands.

The studies included in this thesis are among the first to investigate the diversity and ecology of tussock grassland spider communities from guilds down to species level. I hope that they will motivate other research projects that will try to answer many other questions related to the arachnofauna of New Zealand ecosystems.

Chapter two of this thesis carries out the first evaluation of sampling techniques for spider collection in New Zealand tussock grasslands. A set of sampling methods are compared, and their advantages, disadvantages, and the conditions in which they are most effective are discussed. In ecological research, sampling methods must be selected based on their efficiency in order to maximise available resources (Cardoso et al., 2008; Coddington et al., 1991).

The information obtained from this study allowed the selection of the most efficient sampling methods for the following chapters of the thesis.

Chapter three covers a methodological aspect of ecological studies related to specimen identification through DNA barcoding and taxonomy. The sequencing and analyses of a series of specimens provided evidence of undescribed species and genders, which was critical to classify specimens and complete the data collected and analysed in the ecological studies of the following chapters. Five genera from different families are used to exemplify the potential and pitfalls of DNA technology as a tool for spider identification and extension of ecological data.

Chapter four investigates the drivers of the diversity of spider communities in a native tussock grassland area of Central Otago, in the South Island of New Zealand. Physical and botanical characteristics of the ecosystem and their effects on spider assemblages are assessed through univariate and multivariate analyses of specific guilds, families and species. Trends in community composition and richness revealed affinities related to the preferences or requirements of the different spider taxa. The results of this chapter also uncovered ecological indicator species that could potentially be used in monitoring programs.

Chapter five is the result of collaboration with Dr. Barbara Barratt and her team at AgResearch, Dunedin, who have been carrying out a long term project on the effects of controlled fire on tussock grassland invertebrate fauna. The study included in this thesis examines the changes in the structure of spider communities over time, with data collected before and after fire events. The differences in the effects of spring and summer fires as well as the impact of fire on the dominance of native or exotic species are also discussed.

Chapter 2

Assessing methods for collecting spiders in tussock grasslands

2.1 Abstract

An evaluation of the efficiency of three methods for collecting spider (pitfall traps, suction sampling and foliage beating) in narrow leaved snow tussock (*Chionochloa rigida*) grasslands was conducted in order to determine which one(s) should be given preference and included in monitoring protocols or ecological studies. Pitfall traps were identified as the most effective and efficient method to collect spiders in tussock grasslands. The physical characteristics of tussocks were determined as the main cause of the limited efficiency of other sampling techniques, such as suction sampling. The predominantly vertical structure of tussock plants constrains the living space available to spiders; the thin stems and tips are exposed to the wind and severe climatic conditions. As a result, most spiders are found on the lowest layers of vegetation where the grass blades at the base of the plant are densely packed. Therefore, methods that target these layers will be the most successful for collecting a large proportion of the spider fauna. However, unique species are found through suction sampling and foliage beating. Pitfall trapping should be combined with other techniques when the objective is to carry out an exhaustive spider survey or a complete ecological study of the spider community in tussock grasslands.

Keywords— Sampling methods, spiders, pitfall traps, suction sampling, foliage beating, tussock grasslands.

2.2 Introduction

As one of the major components of global biodiversity, arthropods can provide valuable information for understanding ecological processes and interactions (Wilson, 1987). The study of arthropod diversity has been proposed as a rich data source for different aspects of conservation planning and management, such as reserve selection and design, delineation of distinct biogeographical zones and community types, and early detection of changes in natural areas (Kremen et al., 1993). Therefore, monitoring of key arthropod taxa that are particularly sensitive to environmental changes should be a priority. Effective monitoring requires developing protocols for collecting arthropods based on efficient sampling methods, thus maximising resources (Cardoso et al., 2008; Coddington et al., 1991; Colwell and Coddington, 1994).

Spiders are one of the most appropriate arthropod taxa for monitoring terrestrial invertebrates for a number of reasons. Their collective impact on invertebrate herbivore populations as generalist predators (Riechert, 1974; Riechert and Bishop, 1990) means that they play a key ecological role in most terrestrial ecosystems (Wise, 1993). Spiders respond to changes in habitat characteristics (Riechert and Gillespie, 1986; Uetz, 1991), have high species diversity (Coddington et al., 1996), and are relatively easy to collect and identify.

A small number of studies on arthropods in agroecosystems in New Zealand have highlighted the high numbers of spiders and their possible impact as generalist predators (Clark et al., 2004; McLachlan and Wratten, 2003; Sivasubramaniam et al., 1997; Vink et al., 2004). Also, some ecological research has been carried out on the temporal changes in spider populations in native forests (Alley et al., 2001; Berndt, 1998; Moeed and Meads, 1985, 1986) and on the relationship between invertebrate communities and vegetation cover in shrublands (Derraik et al., 2005). However, interactions of spider communities with other invertebrates and the biological and physical environment in modified and native ecosystems of New Zealand still remain unknown. Furthermore, no research on the efficiency of techniques for sampling spiders in tussock grasslands has been carried out so far.

Grasslands, and mixtures of grass and shrubs, cover nearly 60% of New Zealand's land area (Wardle, 1991), of which around two-thirds are modified

indigenous tussock grasslands (Barratt et al., 2005). Some of these areas of tussock have been retired from grazing and protected since 1983 due to the Protected Natural Area Programme, which protects representative natural areas from around the country (McEwen, 1987). Although the high levels of endemicity and specificity of tussock arthropods and their conservation values have been recognised in New Zealand (McGuinness, 2001), they have attracted limited attention in comparison with plant communities, which have been extensively studied for the last 50 years (Barker, 1953; Dickinson et al., 1992; Grove et al., 2002; Jensen et al., 1997; Mark et al., 2005).

The aim of this study is to provide information on the efficiency of methods for collecting spiders in tussock grasslands by assessing the diversity of spiders collected per sample by each method. Three sampling methods were studied: (1) pitfall trapping, (2) suction sampling and (3) foliage and/or shrub beating in a native grassland area dominated by snow tussocks (*Chionochloa rigida*). Also, additional data from emergence traps were collected during the study. Data collected from the emergence traps were only used in the comparison of the composition of spiders collected by the different sampling methods as the number of emergence trap samples was too low for a complete comparison with the above three methods.

It is expected that this information will be useful for the development of efficient standardised sampling protocols for collecting spiders in tussock grasslands as part of ecological studies or surveys.

2.2.1 Pitfall trapping

Pitfall trapping has been termed as a method for measuring activity-trappability-density (Sunderland et al., 1995). This sampling method is widely used in entomological research for the capture of ground active or epigeal arthropods (e.g., Uetz and Unzicker, 1976, Spence and Niemelä, 1994) because pitfall traps are inexpensive, easy and quick to set up, and capture large numbers of species and rare taxa (Topping and Sunderland, 1992; Ward et al., 2001). There are limitations to pitfall traps, such as their variable efficiency between and within habitats and species (Sunderland et al., 1995) and misrepresentation or overestimation/underestimation of different taxa (Dinter, 1995;

Lang, 2000; Spence and Niemelä, 1994; Topping and Sunderland, 1992) or life stages, as in the case of spider juveniles (Dinter, 1995). Furthermore, several environmental factors may have an effect on catches by affecting spider activity (Dinter, 1995), including temperature, humidity, spatial resistance or animal movement capacity, reproductive behaviour, species interactions and surrounding vegetation (Greenslade, 1964). Nevertheless, pitfall traps are still considered to provide very valuable information (Luff, 1975; Topping and Sunderland, 1992; Uetz and Unzicker, 1976).

2.2.2 Suction sampling

Suction sampling devices have been extensively used in agroecosystems and grasslands for estimating density of arthropods on the ground and in the lowest layers of the vegetation. The effectiveness of this method varies depending on environmental conditions and the structure of the vegetation (Bell et al., 2000; Henderson and Whitaker, 1977; Sunderland et al., 1995), and can underestimate or overestimate certain groups of spiders and other arthropods depending on the their size. Nonetheless, suction sampling is particularly useful when sampling spiders, providing good density estimates (Dinter, 1995) cheaply, easily and rapidly (Bell et al., 2000).

2.2.3 Foliage beating

Beating of foliage is designed for the collection of invertebrates from the foliage or branches of shrubs (Basset et al., 1997; De Castro et al., 2002; Derraik et al., 2001) and can be highly effective when sampling three-dimensionally complex vegetation. The effectiveness of this method can be affected by visibility, number of collectors and environmental conditions. The compact structure of snow tussocks and the presence of shrubs in the sampling sites suggested that this method could be effective and worth assessing in this study.

2.2.4 Emergence traps

This sampling technique has mostly been used for capturing ground emerging invertebrates that show positive phototaxis (Sunderland et al., 1995).

Emergence traps can also be effective for collecting certain spider families (Alley et al., 2001).

2.3 Methods

Sampling was conducted in Ellangowan Scenic Reserve, Banks Peninsula, South Island, New Zealand (43°47.8'S, 173°01.9'E) (Figure 2.1). This 36-ha-reserve represents regionally significant natural features and comprises areas of snow tussock (*Chionochloa* species) and shrubland as well as mixed-broadleaved forest with red beech (*Nothofagus fusca*) and thin-bark tōtara (*Podocarpus totara*). Other main plant species include *Heliohebe lavaudiana*, *Pseudopanax arboreus* and various species of *Coprosma*. The climate of the region is temperate with annual average precipitation and minimum and maximum temperatures of 1650 mm, and 5.4°Cand 13.3°C, respectively (data from adjacent Hinewai Reserve, Banks Peninsula, H. Wilson, pers. comm. 2008).

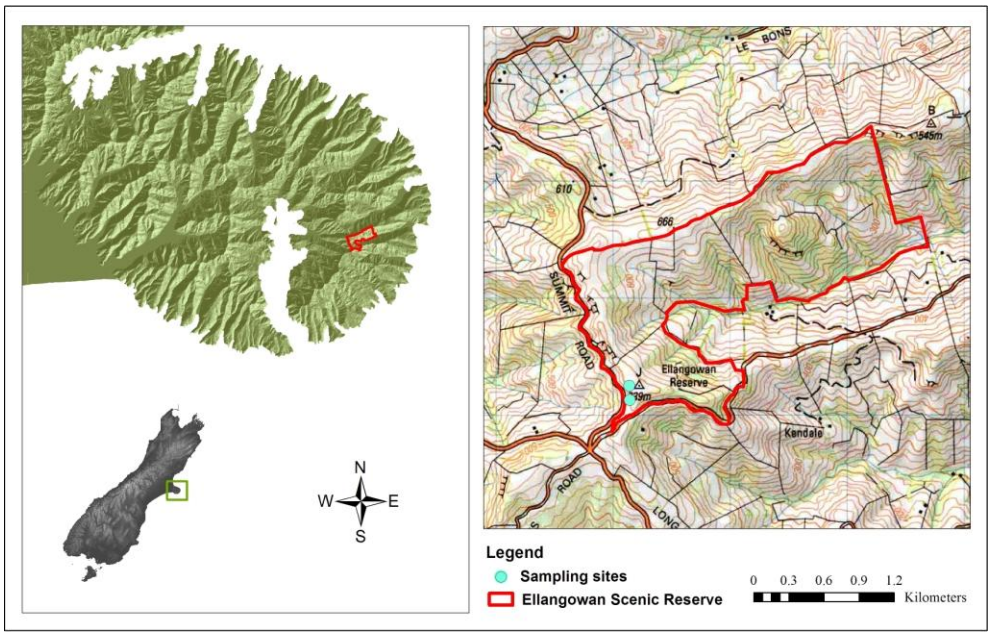

Figure 2.1: Topographic maps of study area. Ellangowan Scenic Reserve, the Banks Peninsula, South Island, New Zealand. Based on map series 1:50,000, Land Information New Zealand, 2003.

Two sampling sites were set up in an area of tussockland/shrubland, with narrow leaved snow tussock (*Chionochloa rigida*) as dominant plant species and scattered *Coprosma tayloriae* and *Pseudopanax arboreus*. The sites were

separated by approximately 80 m and each covered an area of 121 m^2 (11 m x 11 m). Each site was divided into 121 1 m^2 units.

Five pitfall traps were placed in the centre of each site, forming an imaginary 'X', with one trap in each of the four corners and one in the centre (Figure 2.2). The distances between the traps in the corners, and between these and the middle trap were 4 m and 2.8 m, respectively. Each pitfall trap was located in the centre of a 1 m^2 sampling unit and suction and beating samples were collected randomly from the remaining units, excluding the eight 1 m^2 squares surrounding each pitfall trap. This surrounding area was not sampled. As a result, suction and beating samples were collected randomly from a total of 80 squares, each of which covered 1 m^2 (Figure 2.2). This design was selected so that all the samples could be collected from the same area, minimising variability in environmental conditions.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
A	1	2	3	4	5	6	7	8	9	10	11
B	12	13	14	15	16	17	18	19	20	21	22
C	23	24				25				26	27
D	28	29		1		30		2		31	32
E	33	34								35	36
F	37	38	39	40		3		41	42	43	44
G	45	46								47	48
H	49	50		4		51		5		52	53
I	54	55				56				57	58
J	59	60	61	62	63	64	65	66	67	68	69
K	70	71	72	73	74	75	76	77	78	79	80

Figure 2.2: Sampling design in each sampling site. Numbers within circles represent pitfall traps, numbered squares sampling locations for foliage beating and suction sampling, and non-numbered squares the non-sampled area.

Pitfall traps were emptied and five suction and five beating samples collected from each site every two weeks for four months, giving a total of eight sampling times or dates. Locations of previous suction and beating samples were avoided except in the last sampling date when some of them were re-

peated due to the lack of non-sampled squares. Sampling was conducted in the summer of 2006/2007, between November and February in order to cover spiders' most active months.

2.3.1 Pitfall traps

Pitfall traps consisted of a metallic cylinder set into the ground (Figure 2.3) with the rim flush with the ground level. Each cylinder contained a collecting pot, 8 cm in diameter and 9 cm long. A 15 x 15 cm metal roof was placed over each trap to prevent leaves and other plant litter falling into the trap, and rain diluting its content. Each trap contained approximately 150 ml of mono-propylene glycol, which was selected because of its excellent DNA preserving properties (Vink et al., 2005), allowing for molecular analyses of collected individuals in subsequent studies.

Figure 2.3: Pitfall trap; collection pot with mono-propylene glycol, metal roof and metal cylinder (under ground).

2.3.2 Suction sampling

Suction samples were collected with a suction device that consisted of a modified leaf blower with a sampling tube diameter of 16.5 cm (Figure 2.4). Each sample was obtained by placing the suction device in the centre of randomly selected 1 m^2 squares and pressing it against the ground for 10 seconds while it ran at full speed. Samples were placed in pots containing 95% ethanol in order to preserve the DNA of the specimens.

Figure 2.4: Suction sampling; suction sampler and sampling procedure in an exotic grass field.

2.3.3 Foliage beating

Each beating sample was obtained by beating plants located in the centre of each 1 m^2 square with a 77 cm long wooden stick into a fabric tray of 0.70 m^2 held under the sampled plants. Each plant or group of plants was beaten 10 times (Figure 2.5). Two collectors inspected the debris and leaves fallen onto the tray for spiders for approximately ten minutes. Specimens were collected with a brush and placed in vials containing 95% ethanol.

Figure 2.5: Foliage beating; beating of tussocks and subsequent search for spiders on the beating tray.

In addition, one 35 x 35.5 cm emergence trap (Figure 2.6) was set up at site 1. Every two weeks the content of this trap was emptied, and the trap was refilled with mono-propylene glycol and relocated to another sampling square. As a result, a total of eight samples were collected and used to complement the previous methods.

Figure 2.6: Emergence trap.

Samples were taken to the laboratory and stored at -20°C until sorting. Adult specimens were separated and subsequently identified to species, or when not possible, to morphospecies. Taxonomic literature used for spider identification included taxonomic guides by Forster (1967, 1970); Forster and Blest (1979); Forster et al. (1988); Forster and Wilton (1968, 1973), and Vink (2002). All adult specimens were measured and then stored at -20°C. Voucher specimens for all the species and morphospecies collected in the study area are preserved at the Entomology Research Museum, Lincoln University.

2.3.4 Statistical analyses

Four response variables were analysed; (1) number of spider individuals, (2) number of adults, (3) percentage of adults and (4) number of species per sample. Number and percentage of adult spiders were used as indicators of the efficiency of the sampling methods as morphological identification to species of juvenile individuals is difficult and often unreliable (Coddington et al., 1996); hence juveniles are not usually included in studies on spider diversity (Srensen et al., 2002; Toti et al., 2000). For this reason, high numbers and percentage of adults are preferred.

Number of individuals was log transformed and analysed as the response variable in a set of linear mixed effect models. The fixed effects were type of sampling method, the interactions between site (either 1 or 2) and sampling method, and between sampling date and sampling method, whereas the random effect was the interaction between trap number (five within each site) and site. Model selection was based on AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) (Burnham and Anderson, 2002), where models with lower values were favoured.

Because of the large number of zero values in the data of the number of adults per sample, a regression analysis with a multinomial error distribution was used to find significant differences between sampling methods. Values for the percentages of adults per sample required transformation prior to their use in linear mixed effect models. Transformation of percentage values ($sin^2\theta$=(x + 3/8)/(n+3/4)) (Pearce, 1965) allowed their normalisation. Sampling date, method and the interaction between them, and the interaction between site, trap number and sampling date were the fixed and random terms, respectively. A Wald test was then conducted to select terms, retaining the ones whose related Wald statistic provide a χ^2 value that corresponded to $p>0.05$. Subsequent pairwise comparisons between the sampling methods were carried out. Preliminary analyses were carried out with R version 2.9.0 (R Development Core Team, 2009) whereas mixed effect models were built and compared using REML with GenStat 12 (Payne et al., 2009).

Differences in the number of spider species collected by each sampling method were compared through non-parametric estimators Chao1, Chao2, ACE (abundance-based coverage estimator) and ICE (incidence-based coverage estimator) (Chao and Lee, 1992; Magurran, 2004). Chao1 and Chao2 richness estimators consider rare species in their calculation. Such species are singletons (species represented by one individual) and doubletons (species represented by two individuals) in abundance-based samples, or unique species (species present in one sampling unit) or duplicates (species that occur in two sampling units) in replicated incidence data (Chao et al., 2005). ACE and ICE are based on the abundances of species with up to 10 individuals in the sample and species that occur in up to 10 sampling units (Chao et al., 2005, 1993; Magurran, 2004). EstimateS Version 7.5.1 (Colwell, 2006) was used to

calculate species richness estimators that include ACE, ICE, Chao1 and Chao2 (1000 randomizations).

Coleman's rarefaction curves were built using EstimateS to compare the different sampling methods by standardising the number of species to the number of collected samples (Colwell and Coddington, 1994; Gotelli and Colwell, 2001). A series of t-tests (1 sample α=0.05) were conducted between values obtained from the Coleman curves for each sampling method in order to estimate the minimum number of samples at which the difference between methods was significant (Paquin and Dupérré, in prep.).

We used Chao1 in order to calculate inventory completeness, which presents the observed species richness in relation to estimated richness (Cardoso et al., 2008; Srensen et al., 2002). Sampling intensity, defined as the ratio of adult individuals to species, was calculated as a measure of sampling effort (Coddington et al., 1996).

One beating sample was lost during a sampling trip and four pitfall traps were flooded and lost their contents. These samples were excluded from the analyses.

2.4 Results

A total of 235 (plus eight emergence trap) samples collected 311 spider individuals, of which 72 (23%) were adults. Suction sampling yielded the largest number of spiders (145), followed by pitfall traps (107) and foliage beating (39). Emergence traps captured only 20 individuals. Mixed effects models selected using AIC and BIC revealed that sampling method had a significant effect on the number of spider individuals ($F_{2,200}$=30.69, p<0.001). Interactions between site and method ($F_{3,35}$=3.66, p<0.05) (Figure 2.7 and Table 2.1) and between sampling date and method ($F_{21,200}$=1.73, p<0.05) had significant effects on the number of individuals (Figure 2.8 and Table 2.2) for back-transformed means).

Sampling method had a significant effect ($\chi^2_{2,92}$=19.35, p<0.001) on the number of adults. A subsequent pairwise comparison between the sampling methods showed that there were significant differences between all three of them (Pitfall trap>Suction sampling (p<0.01); Suction sampling>Foliage beat-

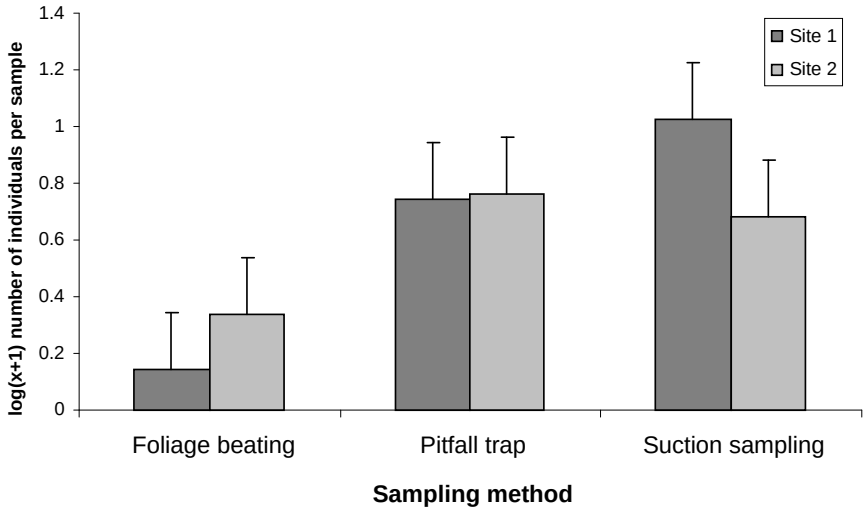

Figure 2.7: Predicted means and least significant differences (Fisher's LSD) of the transformed (log(x+1)) number of individuals per sample, per site and method.

Table 2.1: Back-transformed values of predicted means of the number of individuals per sample (log(x+1)) for each site across all sampling dates.

	Site	
Sampling method	1	2
Beating	2.18	3.07
Pitfall	7.24	7.55
Suction	15.34	6.25

ing (p<0.01), Pitfall trap>Foliage beating (p<0.01)).

There were significant differences in the percentage of adults per sample between sampling methods ($F_{2,32}$=10.81, p<0.001). Pairwise comparisons showed large differences between suction sampling and pitfall traps (t_{32}=3.977, p<0.001), and suction sampling and beating (t_{32}=3.98, p<0.001). However, there were no significant differences between foliage beating and pitfall traps (Figure 2.9 and Table 2.3).

The four sampling methods captured spiders belonging to 18 families and 26 species. Pitfall traps captured 20 species and 16 families, suction sampling 7 species and 4 families, foliage beating 2 species and 2 families and emergence traps 2 species and 1 family. Collected species are listed in Table A.1 of Appendix A.

Non-parametric estimators of species richness also indicated differences

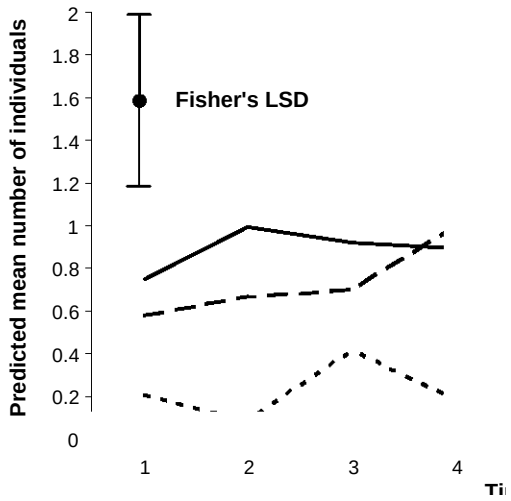

Figure 2.8: Predicted means and least significant differences of the transformed (log(x+1)) number of individuals per sample, for each sampling method and date.

Table 2.2: Back-transformed values of predicted means of individuals per sample (log(x+1)) for each sampling date across both sites.

	Sampling date (sampling time)							
Sampling method	(1) 14-XI 2006	(2) 29-XI 2006	(3) 12-XII 2006	(4) 27-XII 2006	(5) 10-I 2007	(6) 23-I 2007	(7) 7-II 2007	(8) 20-II 2007
Beating	0.23	0.09	0.51	0.20	0.34	0.00	0.34	0.60
Pitfall	1.11	1.70	1.50	1.45	1.78	1.29	0.41	0.34
Suction	0.78	0.95	1.02	1.71	1.17	1.53	2.00	1.99

between sampling methods. Pitfall traps scored the largest values for all the estimators (Table 2.4), followed by suction and beating. Pairwise comparisons between the three methods indicated that the accumulated numbers of species captured by pitfall traps and beating were significantly ($p<0.05$) different after collecting four samples, and after six samples when comparing suction with pitfall and beating (Figure 2.10).

Table 2.3: Back-transformed ($sin^2\theta$=(x + 3/8)/(n+3/4) values of predicted means of the percentage of adults per sample per method.

Sampling method	Adults (%)
Beating	43.04
Pitfall	43.08
Suction	28.67

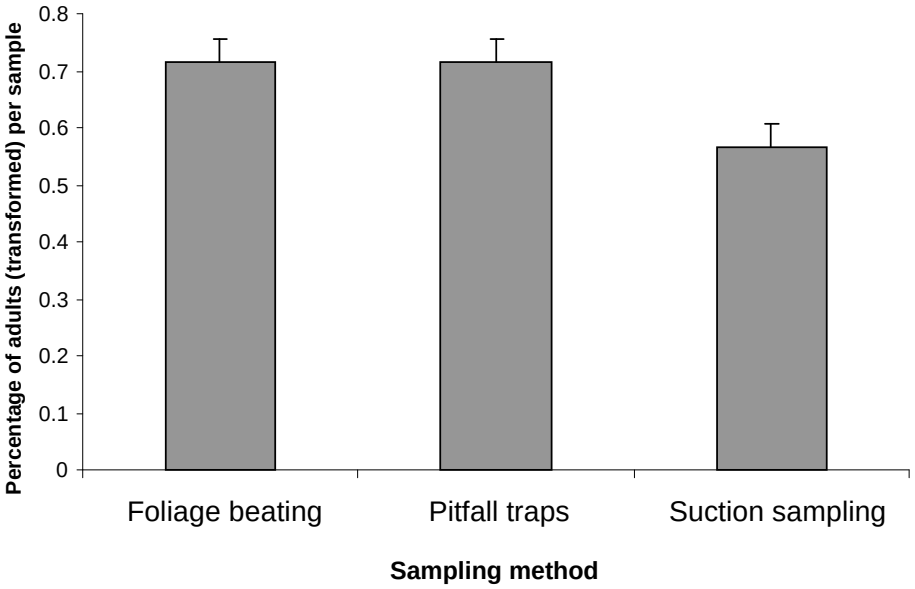

Figure 2.9: Predicted means and standard errors of the transformed ($sin^2\theta$=(x + 3/8)/(n+3/4)) numbers of adults per total number of individuals per sample for each sampling method.

For beating and suction, values of ACE, ICE, Chao1, and Chao2 estimators approximated observed number of species, whereas pitfalls collected much lower numbers than the estimated values (Table 2.4). Coleman rarefaction curves of accumulated number of species over number of suction and beating samples approximated asymptote, while the one of the pitfall samples appeared to be far from reaching it (Figure 2.10). Similar patterns were exhibited by the Coleman curves of pitfall and suction samples over number of collected individuals (Figure 2.11). Beating samples did not show as clear a pattern as those of pitfall traps and suction sampling due the low number of individuals collected by foliage beating.

Levels of completeness achieved by each of the methods differed, with beating scoring the largest value followed by suction sampling and pitfall traps (Table 2.4). Suction sampling had a sampling intensity value of 2.71, pitfalls 2 and beating 1.50.

With regards to the species composition, pitfall traps shared two and three species out of 20 with emergence traps and suction sampling, respectively. The remaining 16 species in pitfall traps and four species in suction samples were

Table 2.4: Number of observed species, singletons, doubletons, non-parametric species richness estimators ACE, ICE, Chao1 and Chao2, and sample intensity and inventory completeness for pitfall traps, suction sampling and foliage beating.

	Observed species	Singletons	Doubletons
Beating	2	1 (50%)	1 (50%)
Pitfall	20	10 (50%)	5 (25%)
Suction	7	2 (28.6%)	1 (14.3%)

	ACE	ICE	Chao1	Chao2	Sample intensity	Inventory completeness
Beating	3	3	2	2	1.5	1
Pitfall	30.2	30.62	27.5	26.34	2	0.72
Suction	7.94	8.08	7.5	7.25	2.71	0.93

unique to those methods. The two species collected through beating were not present in samples collected by other methods (Figure 2.12).

2.5 Discussion

Sample collection methods should be quick, cost-effective, reliable and efficient in order to maximise resources (Colwell and Coddington, 1994). Pitfall trapping has proved to be a very efficient and useful method for collecting terrestrial arthropods in a wide range of ecosystems (Spence and Niemelä, 1994; Uetz and Unzicker, 1976). Our findings indicate that this is also the case when sampling spiders in tussock grasslands, with number of spider individuals and adults per sample being the highest in pitfall traps. Morphological identification of spiders to species is usually possible with adults; hence the importance of collecting large numbers of adults. Pitfall trapping does not only provide information on the size of the spider community but also on the changes in their activity over time (Topping and Sunderland, 1992), which was reflected in the significant effects of the interaction between sampling date and method on the number of spiders.

The effectiveness of pitfall traps and the species that they capture are dependent on their location and surrounding vegetation (Greenslade, 1964). Most of the pitfall traps were placed in inter-tussock spaces, which are prob-

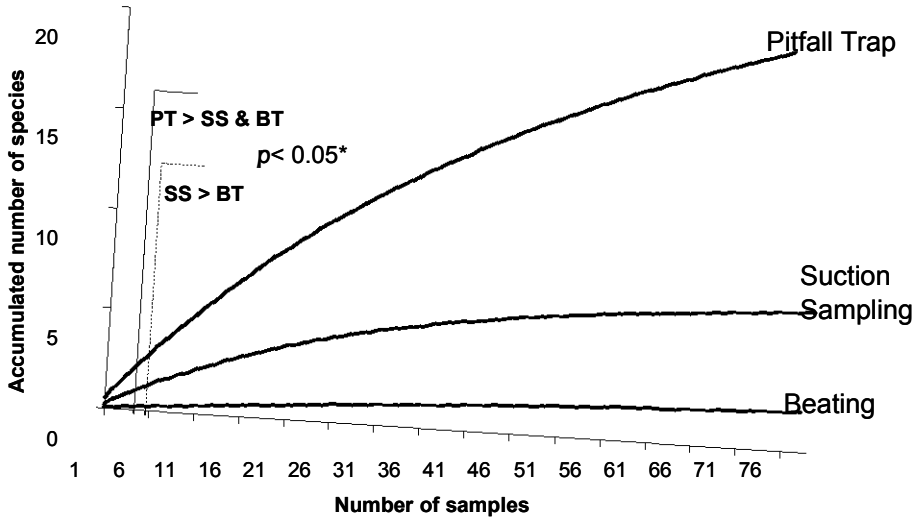

Figure 2.10: Coleman rarefaction curves of the accumulated number of species per sample collected by pitfall traps, suction sampling and foliage beating. PT, SS and BT represent pitfall traps, suction sampling and foliage beating, repsectively.

ably corridors used for hunting by most ground dwelling spiders or as paths between tussocks by web builders. The fact that suction sampling collected significantly fewer spiders than pitfall traps may be related to the structure of the vegetation, which affects the efficiency of this method (Bell et al., 2000; Henderson and Whitaker, 1977). Some tussock species of the genus *Chionochloa* can grow up to 1.5 m, and are very dense at the base of the plant. This is certainly the case for *C. rigida*, which creates microclimatic conditions inside the tussock. These spaces are frequently used by spiders and other arthropods as refuges from unfavourable environmental conditions. The compact base of the tussock would similarly provide spiders protection and substrate to cling to, which may prevent the suction sampler from collecting many of the spiders present. Moreover, quick active spiders may have been underestimated as they may have escaped as collectors approached (Dinter, 1995).

The relatively simple and mostly vertical three dimensional structure of tussocks may account for the low number of spiders collected by beating sampling. The thin and slender leaves of *C. rigida* are not suitable for large or heavy spiders and are only appropriate for the hunting techniques of a limited number of spider families, such as Tetragnathidae, none of which were captured in the study area. Some of the families present in beating samples included

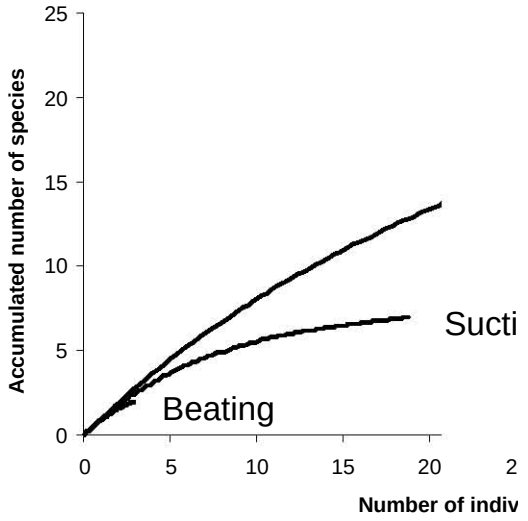

Figure 2.11: Coleman rarefaction curves of the number of species per number of spider individuals collected by pitfall traps, suction sampling and foliage beating.

Salticidae, Thomisidae, Pisauridae, Linyphiidae and Clubionidae. However, all except for the last two families were represented by juveniles.

Of the three methods investigated, pitfalls traps collected the largest number of adults. This is probably due to an underestimation of juveniles by pitfall trapping (Dinter, 1995) as well as their overestimation by suction sampling as suction sampling tends to collect fewer large or heavy specimens (Mommertz et al., 1996), which are frequently adults.

The fact that the rarefaction curves for suction and beating approximated an asymptote (Figure 2.11) indicated that the collection was nearly complete (Coddington et al., 2009). In other words, the numbers of species collected were close to the total number of species that could be captured in the study area by suction and beating. The curves obtained from the pitfall traps appears to be far from reaching an asymptote (Figure 2.11), which suggests that more species may be captured by this method if more traps are used. Therefore, not only did the pitfall traps capture a larger number of species but they could also be capable of capturing even more species provided there was further sampling effort.

Differences in the number of species collected between pitfall traps, and suction samples and beating appeared in early samples. The differences in the

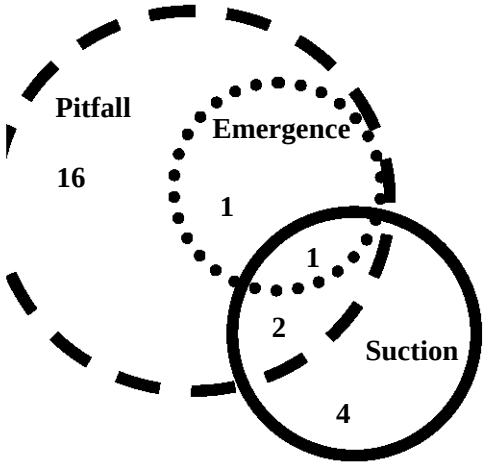

Figure 2.12: Number of species collected and shared by sampling methods.

numbers of species collected for a given number of samples by pitfall traps and beating and suction, and between suction and beating were significantly different just after having collected 4 and 6 samples, respectively (Figure 2.10). This favours pitfall trapping as sampling methods that collect large numbers of species are preferred because of their more accurate estimations of arthropod diversity. Species richness estimators suggested the same conclusions, with estimations for the number of species for pitfall traps much higher than the ones for beating and suction. Furthermore, the considerable differences between the estimated and observed values for pitfall traps indicate that more species would be collected if more effort was applied. The lower value of inventory completeness for pitfall traps also highlighted the need for further pitfall collection in order to obtain a more thorough survey of species present.

Pitfall traps collected the largest number of unique species — in this case defined as species captured only through a specific sampling method — and most of the large and medium-large sized species (Figure 2.12 and Table A.1 of Appendix A). This is probably not only because most of such species belong to ground active hunters that are prone to fall into pitfall traps, but also because of the lack of sufficient power of the suction sampler to aspirate heavy-bodied spiders. This was indicated by the exclusive collection of small species and the proportionally large number of juveniles collected by suction sampling. The absence or limited presence of such spiders at the highest levels of the vegetation would explain why they were rarely collected by beating.

A complete inventory of spiders in an area or ecosystem requires using a range of sampling methods that target different habitats or strata in the vegetation (Brennan et al., 2005). Pitfall traps are designed for collecting ground dwelling animals, whereas suction sampling and beating targets higher layers of the vegetation. The latter two proved to be relatively productive in tussock grasslands as they collected four species (two Mysmenidae, one Theridiidae and one Desidae) and two unique species (one Clubionidae and one Linyphiidae), respectively. Interestingly, three of the four species captured only by suction sampling belonged to web building families and probably live inside tussocks. Many clubionids and linyphiids frequently climb up to the branches of shrubs and other types of vegetation for hunting and for aerial dispersion through ballooning (Wise, 1993), which may explain their presence in tussock leaves and, as a result, in beating samples. Understanding the limitations of these methods is necessary in order to increase the efficiency of sampling protocols (Churchill and Arthur, 1999; Skerl and Gillespie, 1999), and our results suggest that when studying spiders (and probably other arthropods) in tussock grasslands, effort should be concentrated on pitfall trapping. However, complementary sampling methods, such as suction and beating should also be included. Such methods would target specific species or spiders guilds, such as spiders that build webs inside tussocks or hunt on the surface of tussock leaves.

Based on this study, the following recommendations for future spider collection are made:

- Pitfall traps should be included in any study or survey of spider (and probably other arthropod) diversity in tussock grasslands as they are an effective and efficient sampling method.

- More than half of the spider species collected by suction sampling were unique; hence this method could be used as complementary but not necessarily as intensively as pitfall traps.

- Although beating may not be efficient enough to be included in most ecological studies, it could be used in studies targeting certain groups of spiders.

- Other sampling methods need to be assessed in tussock grasslands, such as sweep netting, which can be used in areas lacking shrubs that could damage the net.

2.6 Acknowledgements

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Chapter 3

The use of DNA to complete ecological data

3.1 Abstract

DNA barcoding was launched in 2003 with the aim of specimen identification by using standardised sequence data. Likewise, DNA taxonomy has gained support as a tool for the discovery of undescribed species and stages or genders. Here I assess the potential of this technology for ecological studies through five case studies that exemplify the use of DNA barcoding and DNA taxonomy to complete ecological data by assisting specimen identification and classification, respectively.

Phylogenetic analyses of cytochrome *c* oxidase subunit 1 (COI) sequences discriminated between congeneric species of *Anoteropsis* and recognised undescribed species from the genera *Hypoblemum* and *Laestrygones* as well as the male of the undescribed species *Clubiona blesti*. However, the molecular analyses of two species of the genus *Orepukia* revealed complex intra- and interspecific relationships that are incongruent with morphological identification.

An assessment of the use of generalised mixed Yule-coalescent models for the analyses of COI sequences was conducted. Specimens belonging to morphologically distinctive congeneric species (genera *Orepukia* and *Anoteropsis*) were grouped within the same entities or putative species. These results suggested that the effectiveness of this analysis may depend on the genetic distance between closely related species and the genetic structure of their

populations.

Nevertheless, molecular information can often help identify specimens more accurately, which can improve the quality of ecological data and, as a result, lead to more realistic conclusions. Furthermore, DNA data can provide first indications of complex patterns in inter- and intraspecific molecular diversity caused by geographically structured populations.

Keywords— Spiders, DNA taxonomy, DNA identification, exotic species, undescribed species, indicator species, generalised mixed Yule-coalescent model.

3.2 Introduction

New Zealand is recognised as a biodiversity hotspot (Myers et al., 2000) with high levels of species endemicity. New Zealand spiders have an estimated 97% species endemicity (Paquin et al., 2010). However, a large portion of this diversity has not been described; there are an estimated 1990 species, of which 1126 have been described (Paquin et al., 2010). About 60% of these species are known to science thanks to the work of the late Ray Forster, who described 670 species in over 50 years (Patrick et al., 2000). However, the diversity and ecology of a large proportion of the New Zealand spider fauna remains unknown.

A number of techniques have been developed to assist the discovery of unknown biodiversity. In the last 30 years advances in molecular techniques for genetic analyses have reached the point that it is possible to conduct specimen identification, biodiversity estimations and phylogenetic analyses. Among the many tasks in which DNA technology plays a key role is specimen identification and species delimitation (Tautz et al., 2002, 2003). Microgenomic identification methods use a small fragment of the genome and these have been shown to be useful with species that are impossible to identify morphologically, including viruses (Allander et al., 2001), bacteria (Hamels et al., 2001) and arthropods (Brown et al., 1999; Bucklin et al., 1999), and for the detection of cryptic species (Jarman and Elliott, 2000; Trewick, 2000).

The analysis of DNA has the potential not only to help answer taxonomic and systematic questions, but also to obtain and complement information nec-

essary for ecological studies. For instance, research on traditional community ecology may not be possible with certain organisms without the taxonomic resolution that is only achievable with molecular information. Morphological impediments in the form of extreme or complete resemblance between organisms of different phylogenetic lineages, such as in the case of cryptic species, make DNA technology necessary in certain situations.

3.2.1 DNA data in ecological research

Ecological research has the opportunity to benefit from molecular tools, such as DNA barcoding and DNA taxonomy. Techniques for molecular identification, such as DNA barcoding, have been used to assess microbial species richness and diversity in environmental samples (Gomez-Alvarez et al., 2007; Herrera et al., 2007; Oline, 2006), to detect and identify prey consumed by predators (e.g., Agusti et al., 2003; Hosseini et al., 2008; Monzo et al., 2010) and to estimate ancient biodiversity (Valentini et al., 2009).

Community ecology often requires knowing the number of individuals belonging to each species, family and guild (see chapters 4 and 5), and their relative abundances, in order to analyse the structure of a community. Hence, the accurate identification of individual organisms is necessary to obtain reliable data and interpret it correctly. Identification of specimens, obscured by extreme morphological similarities between congeneric species, can lead to wrong conclusions. For instance, the misidentification of certain specimens can lead to their incorrect classification as native or exotic and, as a result, a misinterpretation of the effects of human disturbances on the native arachnofauna (see chapter 5). As the morphological identification of specimens of certain taxa can be difficult in some circumstances, it may require a multidisciplinary approach that takes into account morphological, behavioural, ecological and genetic information.

Molecular techniques are providing increasingly more information on species delimitation, which allows more accurate estimates of species richness (see chapters 2 and 4), biodiversity patterns at different scales and even the ability to measure the effects of climate change in communities (Gotelli, 2004). Recently, ecological and phylogenetic data have been combined in studies look-

ing at phylogenetic structure of ecological communities (Webb, 2000; Webb et al., 2002, 2006). Molecular identification of organisms for ecological studies or inventories (Monaghan et al., 2009) will become a common practice with advances in technology, such as next-generation sequencing, which has the potential to revolutionise the study and analyses of ecological research (Hudson, 2008; Schuster, 2008; Tautz et al., 2010).

Ecological research on spiders can certainly benefit from DNA identification. Association of specimens of different sexes that belong to undescribed spider species is often an arduous task, especially when they display strong sexual dimorphism (Janzen et al., 2005). The difficulty of the identification of juvenile stages also exemplifies the limitation of relying solely on morphological information with spiders. Given the abundance of juvenile spiders captured in ecological studies, methods that allow their identification would increase and improve the collected data. The inclusion of juveniles in ecological analyses would be particularly useful when sampling techniques are biased towards capturing a greater proportion of juveniles (e.g. suction sampling) (see chapter 2). Molecular information, and more particularly DNA taxonomy and barcoding, has the potential to support and increase the data used in ecological studies (Frezal and Leblois, 2008; Pfenninger et al., 2007) by sorting unidentified genders and juvenile specimens. Juvenile stages have already been matched with morphologically identified adults in other arthropods, including Coleoptera (Ahrens et al., 2007; Caterino and Tishechkin, 2006) and Lepidoptera (Hebert et al., 2004a) through these techniques.

3.2.2 DNA taxonomy and DNA barcoding

Whereas DNA taxonomy is the definition and separation of species through genetic analyses considering evolutionary species concepts based on phylogenetic relationships between organisms, DNA barcoding consists of the use of standardised sequence data for identification of specimens by using a reference database (Ratnasingham and Hebert, 2007; Schindel and Miller, 2005; Vogler and Monaghan, 2007).

DNA taxonomy is gaining support particularly among evolutionary biologists because of its potential to delimit morphologically problematic organisms

and uncover cryptic species (Heinrichs et al., 2009; Leliaert et al., 2009; Monaghan and Sartori, 2009; Neres et al., 2010; Papadopoulou et al., 2009a). Although the term "DNA barcoding" was first used in 1993 (Arnot et al., 1993), it was not until a decade later when this initiative became global (Hebert et al., 2003a,b). One of the principles of DNA barcoding is the use of a standardised gene region (Hebert et al., 2003b) that is present in most organisms and shows little intraspecific but enough interspecific variation to allow discrimination between different species (Hebert et al., 2004b). The favoured fragment by the creators of the DNA barcoding project constitutes 648 bp at the 5' end of the cytochrome *c* oxidase subunit 1 (COI) gene in the mitochondrial DNA (mtDNA).

Several characteristics of COI make it an appropriate standard region for DNA barcoding; first, mitochondrial DNA evolves at a higher rate than nuclear DNA (Brown et al., 1979; Moore, 1995), which increases the differences and diversity of sequences across taxa (Hebert et al., 2003b). Also, as coalescence occurs more rapidly, reciprocal monophyly is achieved sooner in speciation processes (Zink and Barrowclough, 2008). Second, a few sets of universal primers appear to be sufficient to obtain the target sequence in a wide range of organisms (Hebert et al., 2003a). Third, haploid inheritance of mtDNA (Saccone et al., 1999) reduces possible wrong interpretations caused by genetic recombination. And fourth, insertions or deletions (indels) are rare in COI (Barrett and Hebert, 2005) and therefore, sequence alignment is relatively easy (Hebert et al., 2003a). In addition, COI has proved to be useful for other purposes, such as phylogenetic studies (Cox and Hebert, 2001; Wares and Cunningham, 2001).

The potential uses of DNA barcoding have been highlighted by several studies over the last decade (Janzen, 2004). Conservation biology can benefit from the capacity of this technique to identify species (Stoeckle, 2003). Molecular identification of taxa from faeces (Tollit et al., 2009; Valentini et al., 2009), stomach contents (Symondson, 2002), or consumer products (Palumbi and Cipriano, 1998) have proved to be a useful method for the protection of endangered or detection of dangerous species (Rastogi et al., 2007; Vargas et al., 2009). Furthermore, DNA barcoding, combined with DNA taxonomy, could assist ecological studies by identifying individuals of certain organisms,

such as spiders, which sometimes show genitalic polymorphism (Jocque, 2002) and whose juvenile stages are seldom morphologically identifiable (Coddington and Levi, 1991).

3.2.3 The debate

Both DNA barcoding and DNA taxonomy have created some debate over their reliability and correct use. Most criticisms of DNA taxonomy have come from taxonomists concerned about morphology being replaced with DNA information. In this context, they fear that taxonomy could be simplified to one or few DNA sequences (Lipscomb et al., 2003; Seberg et al., 2003). Objections often arise from the long standing debate about the species concept, which often differs between supporters and opponents of DNA taxonomy, and issues related to its use and applicability (Hey, 2001). Other criticisms come from concerns about loss of funding for already underfunded traditional taxonomy in favour of molecular taxonomy.

It has also been argued that the often-used mtDNA divergence is not enough for species delimitation (Moritz and Cicero, 2004; Wheeler, 2004; Wheeler et al., 2004) and that there are often differences between morphologic and molecular assignments of individuals to species (Smith et al., 2005). Hence it has been recommended using molecular information along with other tools for taxonomy (Silva-Brandao et al., 2009).

One of the most contentious issues of DNA barcoding is its supposed applicability to all organisms (Godfray and Knapp, 2004; Moritz and Cicero, 2004; Prendini, 2005; Stoeckle, 2003). As DNA barcoding only works satisfactorily when the taxa are well known, comprehensive taxonomic knowledge of the taxa under study is necessary is most cases (Meyer and Paulay, 2005) and different criteria should be used depending on the taxa (Blaxter, 2003). Hybridisation and introgression between closely related species can hinder the correct identification of organisms when using DNA with maternal inheritance, such as mtDNA, as only one of the two parental species may be identified (Glemet et al., 1998; Hebert et al., 2003a, 2004a). Scepticism has also been cast over the difficulties posed by recently diverged species and the persistence of post-speciation ancestral polymorphism, and the use of multiple genes has been

recommended (Mallet and Willmott, 2003) to prevent incorrect conclusions drawn from identical sequences found in close species (Mallet and Willmott, 2003; Moritz and Cicero, 2004).

Supporters of DNA taxonomy argue that species delimitation based on DNA sequences is possible through formal analyses (Pons et al., 2006b; Sites and Marshall, 2003; Wiens and Penkrot, 2002) or that molecular data can often be a starting point or a way to recognise taxa that might need taxonomic revision and to which further data can be added (Hebert and Gregory, 2005; Smith et al., 2005). DNA barcoding has proved useful in several taxa (Lepidoptera (Hebert et al., 2004a; Janzen et al., 2005), spiders and carabids (Greenstone et al., 2005), mayflies (Ball et al., 2005), crustaceans (Costa et al., 2007), mosquitoes (Kumar et al., 2007), Hemiptera (Wang and Qiao, 2009), Collembola (Hogg and Hebert, 2004)), very accurately (95%) in some cases (Hebert et al., 2004b; Janzen et al., 2005). Certainly, DNA information can complement morphological data (Blaxter, 2004; Hebert et al., 2003a) and may even be crucial when morphology is of no help, e.g. with unidentifiable samples (faeces, processed material, etc.) or when different life stages can not be matched. In these cases, DNA data can help discover cryptic species (Burns et al., 2008; Gomez et al., 2007; Handfield and Handfield, 2006; Pfenninger et al., 2007; Smith et al., 2006).

3.2.4 Analyses of DNA data

Although divergence thresholds have been suggested as an appropriate way to delimit intra- and interspecific variation among species and consequently to assign individuals to species (Hebert et al., 2003a, 2004a, 2003b), more recently their utility has been questioned (Frezal and Leblois, 2008; Hickerson et al., 2006; Meyer and Paulay, 2005). Some of the criticisms are based on the variability of the threshold across different taxa, genes and loci (Ferguson, 2002; Moritz and Cicero, 2004; Seberg et al., 2003; Stoeckle, 2003), errors when divergence time is recent (Wiemers and Fiedler, 2007) and the absence of a "barcoding gap" between different taxonomic ranks (Robinson et al., 2009). Hence a number of alternative analytical methods, including phylogenetic methods and Yule-coalescent methods (GMYC) (Monaghan et al., 2009;

Pons et al., 2006a) have been suggested.

Despite the initial debate, DNA barcoding is considered to have the potential to provide useful information and become another tool for specimen identification (Prendini, 2005). Since not all genes and loci fulfil the requirement of constant and non-overlapping intraspecific and interspecific genetic variation (Lipscomb et al., 2003), a multiple gene/locus approach has been suggested (Blaxter, 2003, 2004; Silva-Brandao et al., 2009). As for species delimitation and phylogenetic analyses, DNA data alone may not be appropriate (Ferguson, 2002; Tautz et al., 2003), but it can add further information to other sources (morphology, ecology, behaviour, etc.) (Burns et al., 2008; Dayrat, 2005; Schlick-Steiner et al., 2006; Sites and Marshall, 2003; Will et al., 2005) and promote the collaboration between experts in different disciplines (Hebert and Barrett, 2005).

3.2.5 DNA barcoding and taxonomy of spiders

There have been attempts to test the efficiency of DNA barcoding in assigning spider individuals to species (Barrett and Hebert, 2005; Hebert et al., 2003a). Barrett and Hebert (Barrett and Hebert, 2005) found no overlap between mean intraspecific and interspecific divergences, with 16.4% between congeneric species and 1.4% between conspecific individuals. A divergence threshold of 4% placed 96% of the individuals in the correct species (identified morphologically) and a threshold of 2% would have correctly placed 100% of them. DNA barcoding was, therefore, considered appropriate for specimen identification in local assemblages because most diversity occurs allopatrically, although the authors have been criticised for not addressing this issue (Prendini, 2005). A later study that included a larger sample size showed that although divergences varied across different families and genera, a large proportion of species had an inter/intraspecific gap, with an average minimum interspecific divergence of 2.61% within genera (Robinson et al., 2009).

Analyses based on DNA taxonomy have been used in studies of spider populations as well as for species delimitation (Bond et al., 2001; Vink et al., 2008). Studies that have attempted to use COI as well as 16S for species delimitation of certain species of spiders, however, did not find an obvious gap

for either gene (Huber and Astrin, 2009).

3.2.6 Aim of the study

The main objective of this chapter is to help complete the data obtained from the samples collected for the studies in chapter 4 and chapter 5. As many collected specimens belonged to undescribed species, their correct classification required combining morphology with molecular data. The conclusions obtained in this study were, therefore, used for the classification of specimens into different morphospecies prior to the ecological analyses conducted in the following two chapters. Here and in the rest of the thesis, the species concept used is based on the phylogenetic species concept, as in Wheeler and Platnick Wheeler and Platnick (2000), and the ecological species concept (Valen, 1976; **?**). A species is here considered as the smallest aggregation of populations diagnosable by a unique combination of character states, and it is assumed that the individuals that belong to this aggregation represent a separately evolved lineage and occupy the same niche or adaptive zone in an ecosystem. Therefore, separate species or morphospecies are assumed to have different ecological roles in the studied tussock grasslands.

Also, this study searches for evidence on the usefulness of molecular information for ecological studies, focusing specifically on DNA taxonomy and barcoding. The present study aims: (a) to contribute to the discovery of new species and unidentified genders through DNA taxonomy, and discuss the repercussions of their detection on conclusions drawn from ecological studies, using species from the genera *Hypoblemum*, *Clubiona* and *Laestrygones* as examples; (b) to add data to the discussion on the usefulness of DNA barcoding for spider species identification through DNA barcoding by using morphologically distinct species (*Anoteropsis hilaris* and *Anoteropsis flavescens*, and *Orepukia orophila* and *Orepukia poppelwelli*); and (c) to provide molecular information on two species that have already proved to be potential indicators of ecological changes (see chapter 4) (*A. hilaris* and *A. flavescens*) by testing various analytical methods for specimen identification and species delimitation that could be used in future ecological studies.

Genus *Hypoblemum* Peckham & Peckham, 1886 (Salticidae)

The genus *Hypoblemum* is known to be native to Australia, with two described species; *Hypoblemum albovittatum* (Keyserling, 1882) and *Hypoblemum villosum* (Keyserling, 1883). *Hypoblemum albovittatum* is present in New Zealand, mainly associated with human environments (Zabka and Pollard, 2002b). During the sampling conducted in Te Papanui Conservation Park (see chapter 4), some specimens similar to *H. albovittatum* were found. As the genitalic characters did not match those of *H. albovittatum* perfectly, a hypothesis was set to test whether the specimens found (*Hypoblemum* sp.) belong to *H. albovittatum* or a possible native species.

Genus *Clubiona* Latreille, 1804 (Clubionidae)

Clubiona is a genus with worldwide distribution, with 13 species described from New Zealand. The male of two *Clubiona* species from New Zealand have not been described yet; *C. blesti* Forster, 1979 and *C. torta* Forster, 1979 (Forster and Blest, 1979). A male specimen, similar to morphologically identified female specimens of *C. blesti* were found in samples collected in Te Papanui Conservation Park. This study aims to find out if the male specimen belongs to the species *C. blesti* using molecular analyses.

Genus *Laestrygones* Urquhart, 1894 (Desidae)

Laestrygones has a distribution limited to New Zealand (three species), Tasmania (one species), the Chatham Islands (one species) and subantarctic islands (one species). *Laestrygones* species live in shrub and grass, and have been recorded in tussock grasslands (Forster, 1970). Specimens of the species *Laestrygones otagoensis* Forster, 1970 were collected in Te Papanui Conservation Park along with morphologically similar specimens that could not be identified with the available taxonomic bibliography. The unidentified male specimens could be easily distinguished from *L. otagoensis*, unlike female specimens, which were extremely similar to the female of *L. otagoensis*. A hypothesis to test whether the collected specimens belong to two separate entities was tested in this study.

Genus *Orepukia* Forster & Wilton, 1973 (Agelenidae)

Orepukia is a widespread endemic genus composed of 24 species, recorded all over New Zealand except for the northern half of the North Island (Forster and Wilton, 1973). Although *Orepukia* species construct webs under logs and litter, they can be relatively active on the ground. The species *Orepukia orophila* and *Orepukia poppelwelli* (Forster and Wilton, 1973) have a southern distribution, having been collected in areas of Central Otago. Specimens of *O. orophila* and *O. poppelwelli* collected in tussock grasslands of Te Papanui Conservation Park are used to test the use of DNA identification to discriminate closely related species.

Genus *Anoteropsis* L. Koch, 1878 (Lycosidae)

Species of the genus *Anoteropsis* are present in New Zealand, New Caledonia and other parts of Polynesia (Platnick, 2010). This genus of ground active spiders is composed of 23 species (20 species in New Zealand), of which several species are common in grasslands (Vink, 2002). The species *Anoteropsis hilaris* (L. Koch, 1877) and *Anoteropsis flavescens* L. Koch, 1878 live in tussock grasslands and can be used as indicators of vegetation structure (chapter 4). This study will provide molecular data on *A. hilaris* and *A. flavescens*.

This study aims to answer the following specific questions about the genera above:

- Is *Hypoblemum* sp. a different species to *H. albovittatum*?
- Is the *Clubiona* specimen collected the undescribed male of the species *Clubiona blesti*?
- Is *Laestrygones* sp. a different species to *L. otagoensis*?
- Can DNA identification methods discriminate between the species *O. orophila* and *O. poppelwelli*?
- Can DNA identification methods discriminate between the species *A. hilaris* and *A. flavescens*?

3.3 Methods

3.3.1 Specimen collection

Specimens were collected in Te Papanui Conservation Park, Central Otago (see Figure 4.1), during 2007/2008 and 2008/2009 sampling seasons. All details of the specimen collection are presented in the methods section of chapter 4. After collection, specimens were placed in 95% ethanol and kept at -20°C. Specimens of each species were selected from various sampling locations to cover as much genetic diversity as possible. See Table B.1 of Appendix B for complete information on analysed specimens.

3.3.2 Sequence extraction

Between 2–4 legs were removed from each specimen depending on the size of the spider and DNA was extracted using the ZM Genomic DNA II Kit (ZYMO Research, Orange, CA, USA). A target fragment of over 1 Kbp of COI was amplified by polymerase chain reaction (PCR) from each specimen. The target fragment was selected because of its previous use in molecular phylogenetic and systematic studies (Framenau et al., 2010; Hedin and Maddison, 2001; Vink et al., 2009, 2008) and as a DNA barcoding region (Robinson et al., 2009). The primers C1-J-1517-spider (5-AATCATARGGATATTGGAAC-3') (Thomas and Hedin, 2008) and C1-N-2776-spider (5'-GGATAATCAGAATAN-CGNCGAGG-3') (Vink et al., 2005) were used for *Hypoblemum* sp. and *Hypoblemum albovittatum* (Figure 3.1). All *Laestrygones otagoensis* and *Laestrygones* sp. specimens were amplified with the primers C1-J-1517-spider and C1-N-2568 (5'-GCTACAACATAATAAGTATCATG-3') (Hedin and Maddison, 2001) except for two specimens, which were amplified with primers C1-J-1718-spider (5'-GGNGGATTTGGAAATTGRTTRGTTCC-3') (Vink et al., 2005) and C1-N-2568. LCO-1490 (5'-GGTCAACAAATCATCATAAAGATA-TTGG-3') (Folmer et al., 1994) and C1-N-2568 were used for *O. orophila* and *O. poppelwelli* as they proved more effective. For *A. hilaris*, *A. flavescens* and *Clubiona blesti* the primers LCO-1490 and C1-N-2776-spider were used.

PCR amplification was carried out using 0.1 µl of i-*Taq*^{TM} DNA Polymerase (iNtRON Biotechnology, Seongnam, South Korea), 0.8 µl of dNTPs

Figure 3.1: Locations of used forward (red arrows) and reverse (green arrows) primers along COI.

(2.5 mM), 0.2 µl of forward and primers (10 mM), 1 µl of 10x PCR buffer, 6.90 µl of water and 0.8 µl of DNA extract at in a Mastercycler® (Eppendorf, Hamburg, Germany) thermocycler with a cycling profile of 35 cycles of 94°C denaturation (30 s), 48°C annealing (30 s), 72°C extension (1 min) with initial denaturation of 3 min and final extention of 5 min. Excess primers and salts were removed from the PCR products of most specimens with DNA Clean & Concentrator™-5 Kit (ZYMO Research). Sequencing PCR were then carried out with purified and non-purified PCR products in forward and reverse directions using 0.5 µl of BigDye® Terminator (v 3.1) (Applied Biosystems), 2 µl of BigDye® Sequencing buffer (v 1.1/3.1), 0.5 µl of forward or reverse primer, 20 ng of DNA from the purified PCR product and the necessary volume of water to add up to 10 µl in total. The profile used in the Mastercycler® was 1 cycle of 96°C, 25 cycles of 96°C (10 s), 50°C (5 s) and 60°C (4 min), and a final extension of 4°C. The resulting product was then sequenced at the Bio-Protection Centre, Lincoln University, New Zealand. The forward and reverse sequences were assembled, compared and edited with Sequencher™ 4.0 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences of species that were used as outgroups in later analyses were obtained from GenBank (Benson et al., 2010) and aligned with edited sequences using Mega 4.0.2 (Tamura et al., 2007). No insertions/deletions or stop codons were found, suggesting no amplification of pseudogenes.

3.3.3 Analyses

A matrix of genetic distances between congeneric specimens was computed separately for each genus using the Kimura-2-Parameter (K2P) model (Kimura, 1980) with pairwise deletion of missing sites and bootstrap anal-

ysis (1000 replicates). Mean, minimum and maximum values of intra- and interspecific distances were then calculated and presented as barplots as they facilitate the interpretation of possible "barcoding gaps" between species (Astrin et al., 2006), and a neighbour joining tree was created from each matrix. Matrices and trees were computed using the software R version 2.10.0. (R Development Core Team, 2009).

The best fitting model for each set of sequences (grouped according to genus or family) was selected out of 88 possible models with jModeltest 0.1.1 (Posada, 2008) using the Akaike Information Criterion (AIC) (Posada and Buckley, 2004). The parameters for the selected model were then included in a maximum likelihood (ML) analysis, estimated with PhyML v3.0 (Guindon and Gascuel, 2003), with bootstrap analysis of 1000 replicates.

No appropriate outgroup sequences that were close enough to the species under study were available for phylogenetic analyses. However, another set of neighbour joining trees and ML trees were calculated including outgroup sequences of non-congeneric species obtained from GenBank to investigate the effects of these outgroups on the analyses. Although sequences of a suitable congeneric outgroup species (*Anoteropsis ralphi*) were available for *A. hilaris* and *A. flavescens*, trees with additional outgroup sequences from GenBank were created. No phylogenetic trees were built for *Clubiona blesti* as it would have been necessary to have outgroup sequences of very closely related species to analyse the relationship between the female and male specimens of *C. blesti*.

An additional estimation of the COI tree topology was conducted for the genus *Orepukia* through partitioned Bayesian analysis (Brandley et al., 2005) implemented in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). After partitioning the data by codon position, separate models were selected for each position with JModeltest 0.1.1 using AIC as the selection criterion. Two simultaneous and independent analyses were run, each with four heated chains, for 2 x 10^7 generations and sampling every 1000^{th} tree. The initial number of burn-in trees to be discarded was estimated by plotting the average standard deviation of split frequences against the number of samples, establishing 25% as the number at which it stabilises (Figure B.1 of Appendix B). A majority rule consensus tree was then built with the remaining trees in MrBayes. All trees were viewed and edited with FigTree version 1.3.1 (Rambaut,

2006-2009).

A generalised mixed Yule-coalescent model (Pons et al., 2006a) on all the analysed haplotypes was tested against previous individual phylogenetic analyses for each genus or family. The function used assumes interspecific branching following a Yule model or increasing or decreasing speciation rates as they approach the present, and neutral coalescent or increasing or decreasing intraspecific branching rates as the ancestral node is approached. After collapsing haplotypes with ALTER (http://sing.ei.uvigo.es/ALTER/), the most likely model was selected with jModeltest. Obtained parameters were computed in PAUP 4.0b.10 (Swofford, 2002) to create an ultrametric maximum likelihood tree under the assumption of a strict molecular clock. The resulting tree was analysed with the package SPLITS (http://r-forge.r-project.org/projects/splits/) in the software R, optimising a single and multiple thresholds for the transition from inter- to intraspecific branching (Fontaneto et al., 2007; Monaghan et al., 2009; Pons et al., 2006a). The two resulting models were then submitted to a log likelihood ratio test to verify whether a multiple threshold would improve the model. Information about the collection of the sequenced specimens and the GenBank accenssion numbers of the outgroups are presented in Table B.1 and Table B.2 of Appendix B.

3.4 Results

3.4.1 Genetic divergence

Intraspecific and interspecific genetic divergences varied across the analysed genera. Intraspecific divergence ranged from 0% to a maximum of 3.43% (Table 3.1). This maximum value corresponded to *Laestrygones otagoensis*. Mean divergence between putative species of the same genus was always greater than within species, except for the two *Orepukia* species. There was no overlap between intraspecific and interspecific divergence in *Hypoblemum*, *Anoteropsis* and *Laestrygones*, with maximum values of the former never approaching the minimum values of the latter (Figure 3.2). The genetic divergence within *Orepukia orophila* was between 0-2.09% and between *O. orophila* and *O. poppelwelli* was 1.36-2.72%, which meant that there was no gap between intraspecific and interspecific divergences between the two species. The sequences of the five specimens of female *Clubiona blesti* and the putative male had a mean genetic divergence of 1.78%, with a minimum of 0% and maximum of 3.18%.

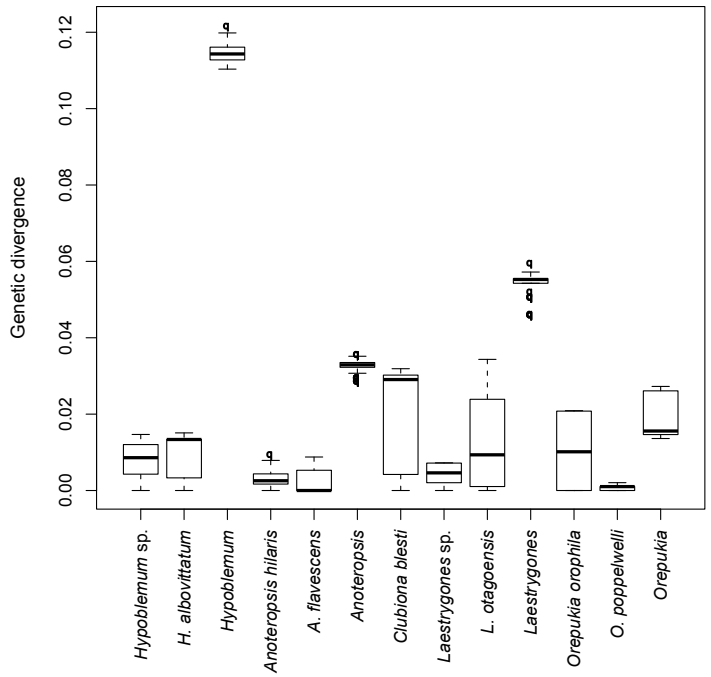

Figure 3.2: Intraspecific and interspecific genetic divergence in COI sequences of the analysed taxa.

Table 3.1: Intraspecific and interspecific genetic distances of analysed taxa.

Species (sample size)	Intraspecific (%) (minimum and maximum)	Interspecific (%) (minimum and maximum)
Hypoblemum sp. (10) *Hypoblemum albovittatum* (6)	0.77 (0-1.46) 0.9 (0-1.5)	11.47 (11.03-12.18)
Clubiona blesti (6)	1.78 (0-3.18)	n/a
Laestrygones otagoensis (9) *Laestrygones* sp. (4)	1.26 (0-3.43) 0.42 (0-0.72)	5.39 (4.62-5.97)
Orepukia orophila (9) *Orepukia poppelwelli* (10)	1.04 (0-2.09) 0.07 (0-0.2)	1.88 (1.36-2.72)
Anoteropsis hilaris (10) *Anoteropsis flavescens* (10)	0.32 (0-0.96) 0.26 (0-0.87)	3.28 (2.88-3.59)

3.4.2 Phylogenetic analyses

Maximum likelihood trees with and without outgroups showed similar patterns, with the former being based on more complex models overall. Table B.3 of Appendix B contains models selected for all neighbour joining, ML, Bayesian and GMYC analyses. Maximum likelihood trees without outgroups and all trees with outgroups can be found in Appendix B (Figure B.2–Figure B.13).

Genus *Hypoblemum* (Salticidae)

Approximately 1,200 bp of the COI gene were sequenced from six specimens of *Hypoblemum albovittatum* and 10 of *Hypoblemum* sp. Neighbour joining and ML trees with and without outgroup species clustered all the specimens identified as either *Hypoblemum albovittatum* or *Hypoblemum* sp. into two distinct separate clades (Figure 3.3, and Figure B.2, Figure B.3 and Figure B.4 of Appendix B). The neighbour joining and ML trees that included sequences of *Marpissa pikei* and *Trite planiceps*, two Australian species, as outgroups showed a similar relationship between the two *Hypoblemum* clades (Figure B.3 and Figure B.4, of Appendix B). Bootstrap supports for the branches grouping the specimens of *Hypoblemum albovittatum* and *Hypoblemum* sp. were 100% in the neighbour joining trees without and with outgroups (Figure 3.3 and Figure B.3 of Appendix B).

The GMYC model considered the two *Hypoblemum* species as separate

entities (Figure 3.9).

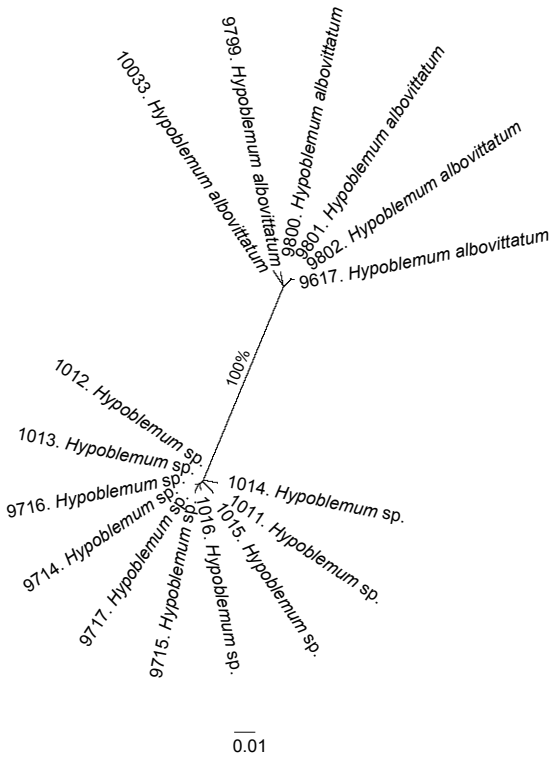

Figure 3.3: Unrooted neighbour joining tree of *Hypoblemum* sp. and *H. albovittatum* specimens. The percentage value represents bootstrap support.

Genus *Clubiona* (Clubionidae)

Five female specimens and the putative male of *Clubiona blesti* were sequenced and 1,200 bp long fragments obtained. The lowest genetic divergences between the male individual (specimen 1026) and the female specimens were 0.65%, 0.59% and 0.85% for specimens 1024, 1025 and 1027, respectively (Table 3.2). For the GMYC analyses, GenBank sequences of *Castianeira* sp. and *Clubiona bishopi* (Clubionidae) were used as they were the phylogenetically closest species to *C. blesti* among the available sequences. The GMYC tree clustered the male individual within the group of female individuals identified morphologically as *C. blesti*, with very little genetic distance between them (Figure 3.9).

Genus *Laestrygones* (Desidae)

Sequences of >900 bp were obtained from nine specimens of *Laestrygones otagoensis* and five *Laestrygones* sp. that had been morphologically identified

Table 3.2: Intraspecific genetic divergence in COI sequences of female specimens of *Clubiona blesti* and the suspected male.

	1021 ♀	1022 ♀	1024 ♀	1025 ♀	1027 ♀	1026 ♂
1021 ♀	0%	0.08%	3.09%	2.99%	3.19%	2.96%
1022 ♀		0%	2.98%	2.91%	3.10%	3.05%
1024 ♀			0%	0%	0.11%	0.65%
1025 ♀				0%	0.25%	0.59%
1027 ♀					0%	0.85%
1026 ♂						0%

as possibly different species. Trees with and without the outgroup sequences of *Badumna longiqua* placed the two species of *Laestrygones* into two separate clades (Figure 3.4, and Figure B.5, Figure B.6 and Figure B.7 of Appendix B), each of which showed different degrees of intraspecific genetic variation. Greater genetic diversity was found in *Laestrygones otagoensis* than within *Laestrygones* sp., which is shown by the two clans within the former (Figure 3.4). High bootstrap values of 100% supported the separation of these two taxa in the neighbour joining trees with and without outgroups (Figure 3.4 and Figure B.6 of Appendix B).

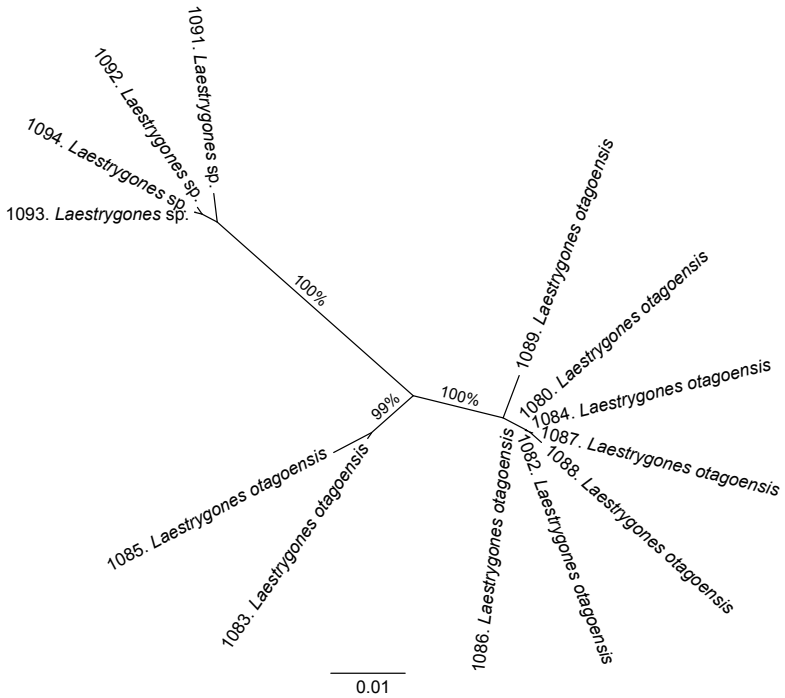

Figure 3.4: Unrooted neighbour joining tree of *Laestrygones* sp. and *L. otagoensis* specimens. Percentage values represent bootstrap supports.

The results of the neighbour joining tree were concordant with the GMYC analysis, which considered the two species of *Laestrygones* as distinct entities (Figure 3.9).

Genus *Orepukia* (Agelenidae)

Approximately 1,000 bp were obtained from eight specimens of *Orepukia orophila* and ten of *O. poppelwelli*. A neighbour joining tree separated the specimens of the two species into three clans (Figure 3.5). This was confirmed by the maximum likelihood analyses, which created a similar tree (Figure B.8 of Appendix B). Both analyses gave bootstrap support values greater than 90% for the three clans. The Bayesian analysis of the same sequences also indicated the existence of three clans, with values of 100% for the posterior probability (Figure 3.6). The neighbour joining and ML trees that included the outgroup sequences of *Novelena intermedia* and the New Zealand species *Neoramia janus*, also created three distinct ingroup clades (Figure B.9 and Figure B.10 of Appendix B).

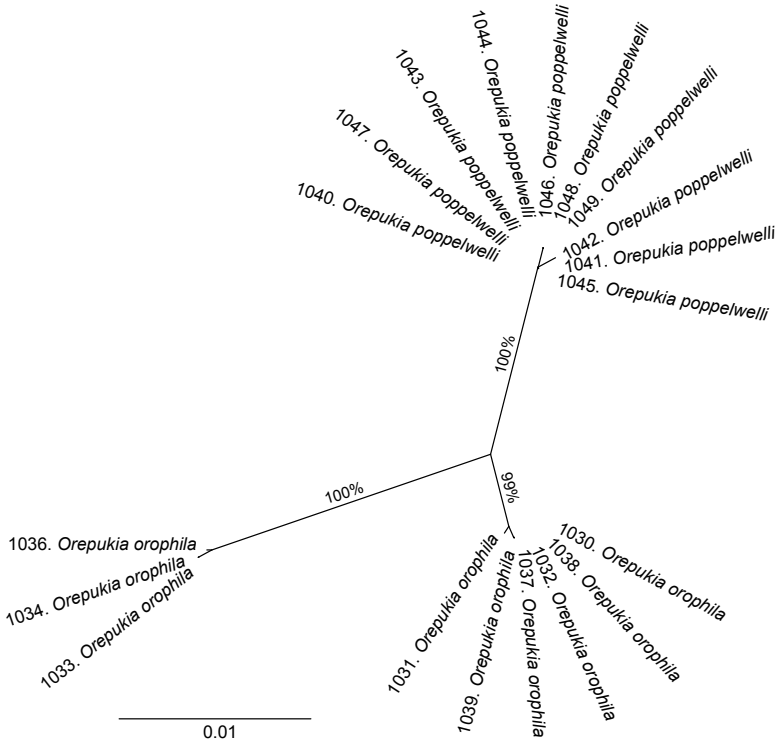

Figure 3.5: Unrooted neighbour joining tree of *O. orophila* and *O. poppelwelli* specimens. Percentage values represent bootstrap supports.

Unlike the other phylogenetic analyses, the GMYC model grouped all the

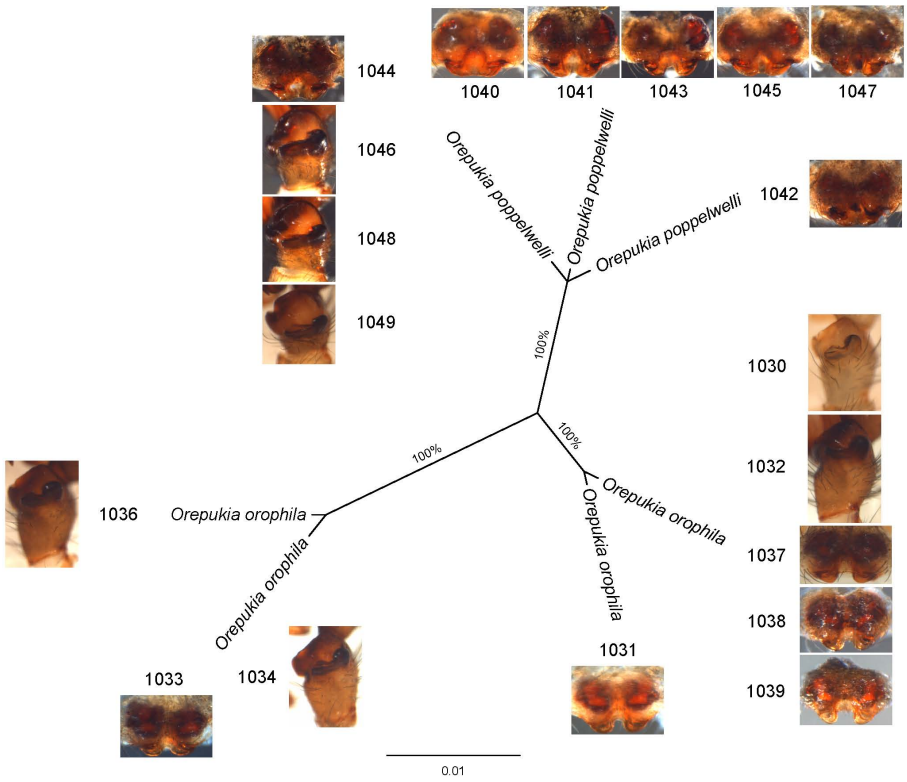

Figure 3.6: Bayesian consensus tree of the sequences obtained from all *Orepukia* specimens. Photos of the ventral view of the epigynes of the females and the lateral tibial apophyses of males are placed next to their corresponding specimen names.

specimens of *Orepukia* in a single clade or species (Figure 3.9). The GMYC model with multiple thresholds clustered *Orepukia* specimens into two clades, one with specimens 1036, 1033 and 1034 another with the remaining specimens of *O. orophila* and O.*poppelwelli*.

Genus *Anoteropsis* (Lycosidae)

Over 1,100 bp long sequences were obtained from 10 specimens of each species of *Anoteropsis*. Both neighbour joining and ML trees showed graphically a clear separation between the *A. hilaris* and *A. flavescens* as two different species (Figure 3.7). The two groups of specimens belonging to *A. hilaris* and *A. flavescens* in the ML tree had bootstrap branch support values of 99% (Figure B.11 of Appendix B). The trees including the outgroups *Artoria separata* and *Schizocosa rovneri*, showed an identical relationship between *A. hilaris* and *A. flavescens* (Figure B.12 and Figure B.13 of Appendix B).

The GMYC models with single and multiple thresholds considered the

specimens of *A. hilaris*, *A. flavescens* and *A. ralphi* as a single species (Figure 3.9).

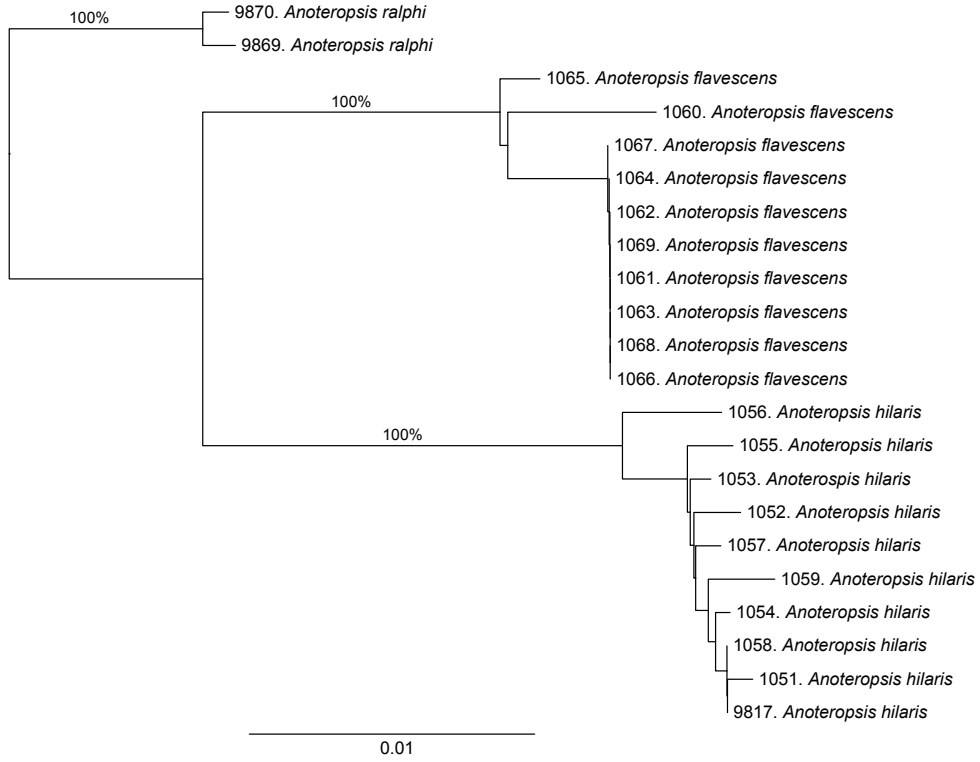

Figure 3.7: Neighbour joining tree of *A. hilaris*, *A. flavescens* and the outgroup *A. ralphi* specimens. Percentage values represent bootstrap supports.

GMYC model

The generalised mixed Yule-coalescent (GMYC) model with a single threshold was favoured against the multiple threshold model as the latter did not improve the model significantly according to the log likelihood ratio test (χ^2=1.80287, p<0.01). The threshold time between speciation and coalescence nodes was set at -0.0211 (Figure 3.8), at which point there was a steep decrease in the likelihood value in the lineage-through time plot (Figure 3.8). The change in branching rates from inter to intraspecific nodes resulted in 22 identified distinct entities or putative species (Figure 3.9), with the confidence interval of 95% extending it between 20 and 24.

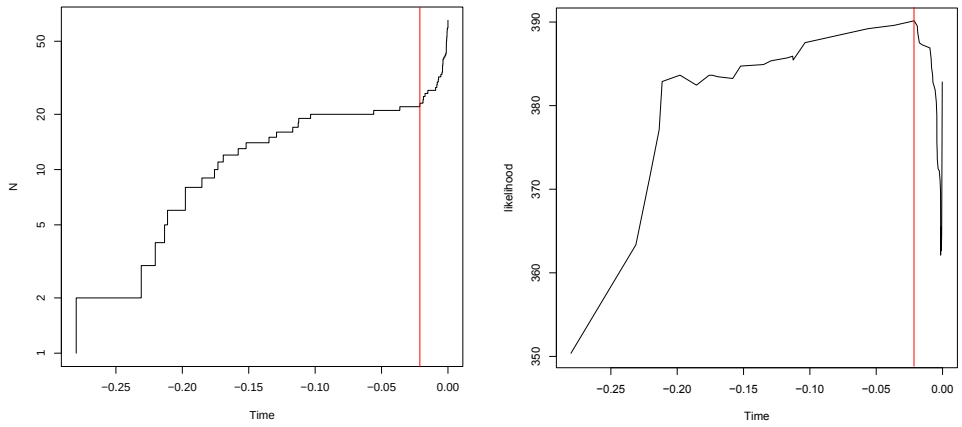

Figure 3.8: Log-lineage through time plot and log likelihood surface plot. The clock-calibrated tree that these plots were based on was used to create a GMYC model with a single threshold of all haplotypes. The red line indicates a steep increase in the transition from interspecific to intraspecific branching.

3.5 Discussion

3.5.1 Recognition of a native species: *Hypoblemum*

Inter- and intraspecific divergence distances and phylogenetic analyses through neighbour joining and maximum likelihood trees indicated that *Hypoblemum* sp. is a different putative species to *H. albovittatum* despite their extreme morphological resemblance. Recognition or identification based on morphology can underestimate true evolutionary diversity (Bond et al., 2001) as characters such as genitalia may evolve slower than previously thought (Hedin, 1997), which may be the case with *Hypoblemum* sp. and *H. albovittatum*. The Salticidae is a family well known for the difficulties of morphological identification and abundance of cryptic species (Paquin et al., 2010), which may explain why only 49 species of this family have been described or recorded in New Zealand, out of an estimated 200 (Zabka et al., 2002).

Hypoblemum albovittatum is thought to be native to Australia and present in New Zealand, associated with human environments. Its distribution is probably limited by temperature, being present in the North Island and northern South Island (Zabka and Pollard, 2002b). The individuals of *Hypoblemum* sp. collected in Te Papanui Conservation Park are present in a much more southerly location than the known distribution of *H. albovittatum*. Given the remoteness and high altitude (app. 1,100 m amsl) of the recorded population

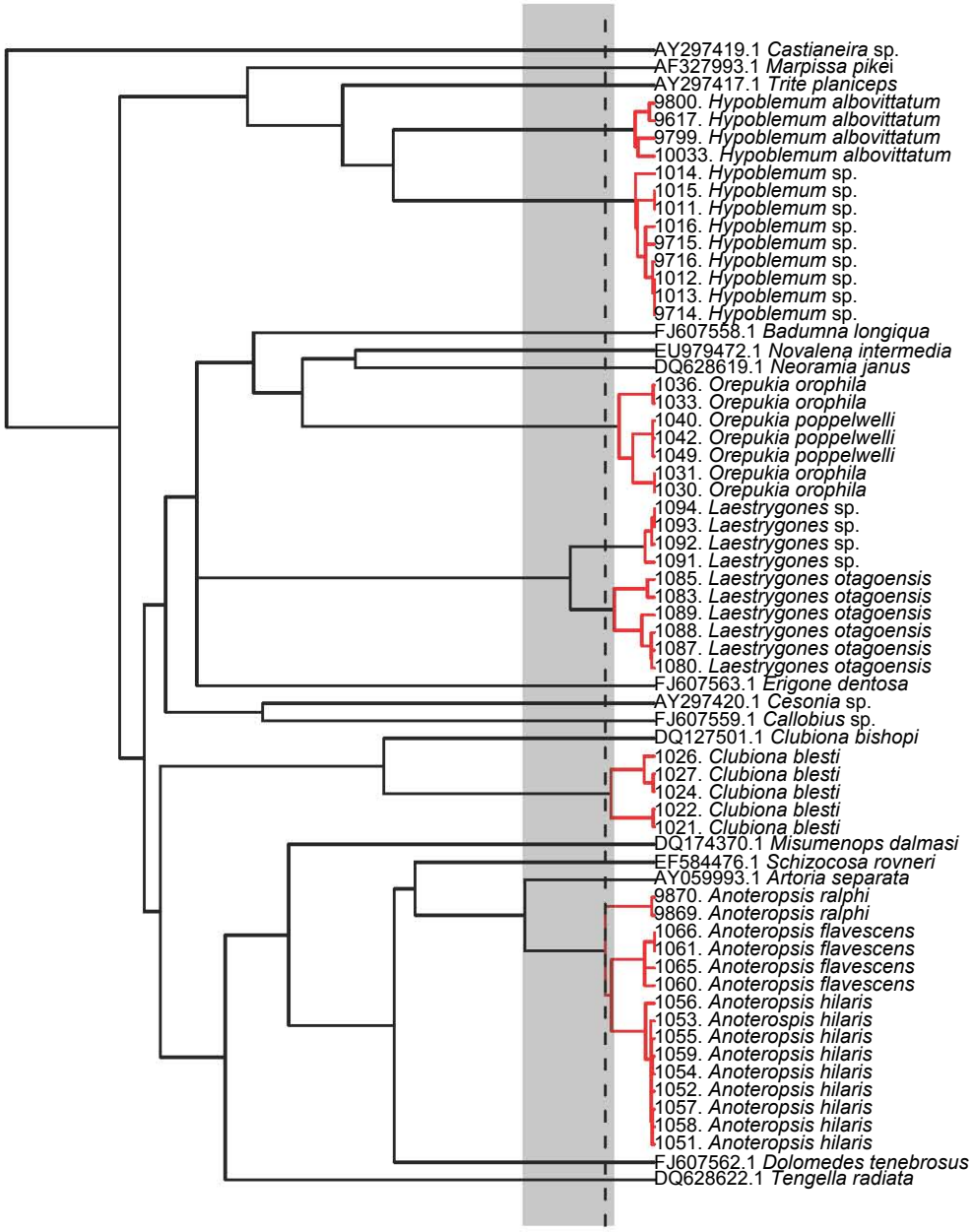

Figure 3.9: Generalised mixed Yule-coalescent model of all sequences. Red lines indicate intraspecific branches estimated in the likelihood procedure. The threshold is represented by the dashed line and the confidence interval by the grey area.

of *Hypoblemum* sp., it is sensible to infer that this species is native, probably endemic, to New Zealand and adapted to colder conditions. The occurrence of other species of *Hypoblemum* in such areas is consistent with the idea that other species and genera of the same subfamily (Euophryinae) are present in New Zealand (Zabka and Pollard, 2002a). Including sequences of such species as outgroups would improve the resolution of the analyses carried out in this study and would probably confirm its findings. Unfortunately, the closest available sequences belonged to two Australasian species, which may be phylogenetically too distant from *Hypoblemum* sp. and *H. albovittatum*.

Although New Zealand still has very high levels of spider endemicity, the number and distribution of exotic spider species has increased since the arrival of Europeans in the 19^{th} century. Human activities have had a key role in the dispersal of exotic species and some of them have shown the ability to colonise recently disturbed areas and possibly replace native species (chapter 5). Therefore, the distinction of exotic from native species is crucial for understanding the effects of environmental changes — human induced or not — on the native fauna.

Previous studies have suggested and shown the potential of DNA data for biosecurity through the detection and identification of exotic species (Armstrong and Ball, 2005; Ball and Armstrong, 2006; Greenstone et al., 2005; de Waard et al., 2009). The two species of *Hypoblemum* examined in this study demonstrate that it is necessary to consider tools other than morphology for an accurate identification of specimens and the correct interpretation of ecological data.

3.5.2 Discovery of undescribed genders and species: *Clubiona* and *Laestrygones*

An undescribed gender of a described species and a new putative species were discovered by using sequence data from COI to support limited morphological evidence. As with *Hypoblemum*, the lack of available sequences that are long enough and phylogenetically close enough to the target species limited the phylogenetic analyses, especially in the case of *Clubiona blesti*, for which no adequate outgroup was found. Nevertheless, the analyses conducted on the

data exemplify the potential of molecular technology to detect and identify specimens and complete species counts for ecological studies.

Genus *Clubiona*

The clustering of a suspected male specimen of *Clubiona blesti* among the morphologically identified females and the small genetic divergence between them confirmed its correct identification. Although it may appear that a larger sample size is necessary to consider the unidentified specimen as the male of *C. blesti*, the almost identical sequences and the morphological similarities indicate that it belongs to the same species as the female specimens. The presence of the sequenced specimens in a remote tussock grassland confirmed previous observations about *C. blesti* being associated with tussocks and found in mountain tussock grasslands (Forster and Blest, 1979). The remoteness of such mountain areas may explain why the male of *C. blesti* has not yet been described. Descriptions of species based on a single gender are relatively common among spiders (Coddington et al., 1991), mainly due to insufficient sampling and strong sexual dimorphism (Framenau et al., 2010; Kuntner and Coddington, 2009; Langlands and Framenau, 2010). Examples of species with only one holotype are not rare among the spider species described from New Zealand, and some of them may need to be synonymised (C. J. Vink, pers. comm.).

Genus *Laestrygones*

After slight morphological differences were detected among specimens of *Laestrygones otagoensis*, an analysis of COI sequences of several specimens led to the discovery of an undescribed species, probably closely related to *L. otagoensis*. Although males of the undescribed species could be identified morphologically (Figure 3.10), females were difficult to distinguish. The genetic analyses suggested that the sequenced specimens could be classified as a separate, probably congeneric, species although the hypothesis of its status as a species should be tested by adding further morphological and ecological data (Lipscomb et al., 2003). It is also worth mentioning that the two species show different degrees of genetic diversity, although this may be due to differences in sample size or perhaps a restricted distribution of *Laestrygones* sp. Six species

of *Laestrygones* have been described from Tasmania and New Zealand and for only one out of the five from New Zealand have both genders been described (Forster, 1970). The drawings describing the genitalia of *Laestrygones* species are of limited quality and may lead to confusion. Given these two facts, it would not be surprising that the species named *Laestrygones* sp. belonged to one of the species partially described by Forster.

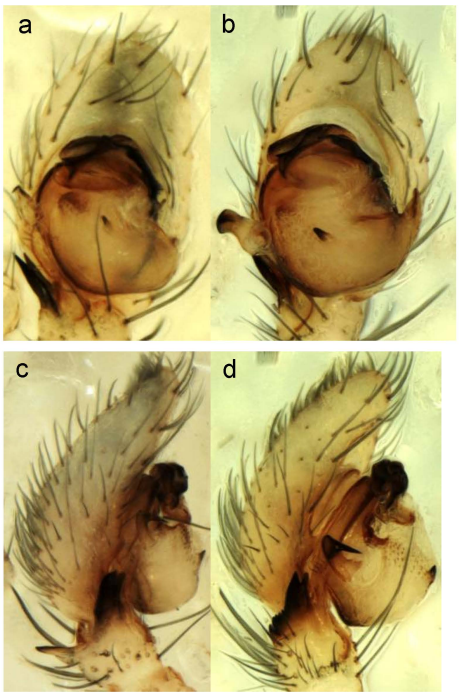

Figure 3.10: Ventral and lateral views of male pedipalp of *Laestrygones otagoensis* and *Laestrygones* sp.: (a, c) *L. otagoensis*, (a) ventral view, (c) lateral view; (b, d) *Laestrygones* sp., (b) ventral view, (d) lateral view.

The examples provided by *Clubiona blesti* and *Laestrygones* demonstrate the potential of DNA taxonomy as a method to help discover elusive or morphologically difficult species and genders. It is expected that descriptions of the male of *Clubiona blesti* — after further genetic analysis including sequences of morphologically similar sister species — and possibly the new species of *Hypoblemum* and *Laestrygones* will be completed and submitted for publication in the near future by the author.

3.5.3 DNA identification of congeneric species: *Orepukia*

The COI sequences of the specimens of *O. orophila* and *O. poppelwelli* analysed here showed differences in intraspecific genetic diversity between the two

species and support the careful use of DNA barcoding for specimen identification. The large intraspecific divergence of *O. orophila*, the small interspecific divergence between the two species and the absence of a barcoding gap between them suggest that a single gene approach may not always be valid for species identification as certain specimens with very similar or different COI sequences could be misidentified. Phylogenetic analyses supported this idea as they revealed three clans, two of which were morphologically identified as *O. orophila*. *Orepukia orophila* and *O. poppelwelli* are morphologically distinct and can be differentiated by their genitalia (Forster and Wilton, 1973). In addition, each species has a different abdominal pattern and body size. The mean body length of *O. poppelwelli* is larger than that of *O. orophila*, with its carapace being 25% larger (Forster and Wilton, 1973). The clear morphological differences between the analysed specimens were not congruent with the clans identified by the genetic analyses of the specimens.

The purpose of this study and DNA barcoding as a whole is not to resolve phylogenetic relationships through methods, such as Bayesian analyses, commonly used in DNA taxonomy. However, a Bayesian analysis was conducted to gain support for the three clans of *Orepukia* and the unexpected interspecific relationship shown by the neighbour joining and maximum likelihood trees.

The two clans of *O. orophila* may be explained by geographic differentiation as specimens of the clan formed by specimens 1033, 1034 and 1036 were found in sites 4 and 5, whereas the remaining specimens were collected in sites 1, 2 and 3. Given the geographical separation (minimum of 4 km) and the differences in the vegetation between the two sets of sites (chapter 4), the genetic divergence between the two clans may reflect a highly structured distribution of genetically distinct populations.

Large genetic distances between intraspecific populations can lead to misinterpretation of DNA data for species identification if morphological characteristics are not considered. A disagreement between morphological and molecular information is certainly possible (Wahlberg et al., 2003) and closely related species have shown very small genetic divergences caused by very similar sequences (Janzen et al., 2005; Kumar et al., 2007; Maddison and Hedin, 2003a; Wiemers and Fiedler, 2007). Using sequence data from Hedin (2001), Robinson (2009) calculated that species of the genus *Hypochilus* (Hypochil-

idae) had an average of 10.86% and a maximum of 17.74% of intraspecific divergence, whereas five species pairs from three genera had interspecific divergence values of 0%. In some species of the family Pholcidae, COI pairwise p-distance between conspecific specimens has been found to have a maximum value of 10.9% whereas the minimum congeneric distance was 8.7% (Astrin et al., 2006).

Low relative genetic distances in COI between morphologically described species, such as the two *Orepukia* studied here, may be caused by insufficient sampling, incomplete lineage sorting (Hebets and Vink, 2007) or incorrect taxonomic classification caused by genitalic polymorphism, which could suggest synonymy (Garb et al., 2004). However, clear morphological differences between the two species suggest that the latter may not be the case. Small genetic similarities may be caused by a recent divergence between species (Hebert et al., 2003a), interspecific gene flow (Funk and Omland, 2003) or hybridisation, given the uniparental inheritance of mtDNA, introgression (Ballard, 2000; Paquin and Hedin, 2004; Sota et al., 2001) or retention of ancestral polymorphism predating species separation. Analyses based on a single gene may not be sufficient for correct DNA identification (Will et al., 2005), as in the case of *Orepukia*, and a multigene approach may be required.

Likewise, in DNA taxonomy, single gene analyses are not likely to resolve complex inter- and intraspecific relationships or the delimitation of species boundaries (Roe and Sperling, 2007). In the case of *Orepukia*, including sequences of closely related congeneric species and incorporating an analysis of rapidly evolving nuclear genes may help understand the inter- and intraspecific diversity patterns of *O. orophila* and *O. poppelwelli*, which should result in a more accurate taxonomic classification. Incorporation of multiple genes/loci and individuals from different locations will improve the performance of DNA identification and validate DNA taxonomy (Frezal and Leblois, 2008; Funk and Omland, 2003; Meyer and Paulay, 2005; Moritz and Cicero, 2004; Scotland et al., 2003; Seberg et al., 2003; Wahlberg et al., 2003; Wiemers and Fiedler, 2007; Will and Rubinoff, 2004).

In conclusion, DNA identification or barcoding of COI has not been successful in assigning *Orepukia* species names to individuals but rather in highlighting the need for a more thorough study of *O. orophila* and *O. poppelwelli*,

and perhaps the entire genus, by incorporating ecological and behavioural information (Paquin and Hedin, 2004).

3.5.4 DNA identification of indicator species: *Anoteropsis*

The use of DNA barcoding with COI proved to be valuable to assign correct species names to 20 specimens of the genus *Anoteropsis*. A large gap between the intra- and interspecific genetic divergences suggested the successful use of this method, which was confirmed by neighbour joining and maximum likelihood trees. However, the GMYC analyses did not recognise the different specimens of *Anoteropsis hilaris*, *A. flavescens* and *A. ralphi* as belonging to separate species (Figure 3.9). The incongruence between the different methods may be due to the unbalanced sampling of haplotypes from different taxa or the different speciation and coalescence branching rates across the analysed taxa.

The present chapter provides valuable information on *Anoteropsis hilaris* and *Anoteropsis flavescens*, two species that proved to be indicators of changes in plant structure in tussock grasslands. Given the success of phylogenetic analyses like simple neighbour joining trees based on COI sequences in discriminating the two species, information of this kind will be useful in future studies that require the identification of morphologically unidentifiable specimens, such as juveniles or fragments found in stomach contents of predators. Future development and adaptation of technologies such as next generation sequencing will provide the opportunity to use similar sequence data to answer specific ecological questions related to community composition and change caused by disturbances or time.

3.5.5 GMYC model for species recognition

The generalised mixed Yule-coalescent model has been suggested as a method to recognise or quantify the number of species in rapid biodiversity assessments of local communities (Monaghan et al., 2009; Pons et al., 2006a). Although limited by the number of taxa, the results of this study suggest that the effectiveness of GMYC may depend on the sampling approach and the distribution of genetic diversity or divergence across the species. Although this

method is considered conservative by some (Papadopoulou et al., 2009b), it appears that recently diverged species, closely related species or species with low interspecific divergence, such as *Anoteropsis hilaris*, *A. flavescens* and *A. ralphi*, may not be recognised as separate species. Large phylogenetic distances between families and genera included in the conducted analyses may have caused the setting of the between/within species threshold too deep into the tree and far from the nodes that separate the three species of *Anoteropsis*. Likewise, species, such as *O. orophila* and *O. poppelwelli*, with no clear genetic separation or "barcoding gap" between inter- and intraspecific divergences caused by a strong geographic structure may hinder the use of GMYC models. As in cases like these methods that rely on differences in fixed diagnostic character states may be more effective (Pons et al., 2006a), consistent unique combination of morphological characters shared by specimens were used as the criteria to classify them as separate species for the ecological analyses carried out in the following chapters. Such classification was backed by the placement of specimens in separate lineages by the other phylogenetic analyses (neighbour joining and maximum likelihood), and therefore congruent with the species concept used in this thesis, except in the case of the *Orepukia* species. The classification of specimens into *O. orophila* and *O. poppelwelli* was based on morphological characters as it was considered that further molecular data was required to confirm the lineages shown by the analyses of COI.

The lack of enough sequences of intermediate taxa may be another reason for obtaining a tree with a topology and threshold skewed towards the tips. As incomplete sampling can affect clustering (Lohse, 2009; Papadopoulou et al., 2009b), the validity of the results of this study could be tested by including more sequences of species placed in intermediate phylogenetic positions, equalising the number of haplotypes per species or including data on other genes/loci (Lohse, 2009; Monaghan et al., 2009). A complete taxon sampling, in turn, depends on a solid understanding of the relationships between the target taxa, which, in the case of the New Zealand arachnofauna, requires further taxonomic and phylogenetic research.

3.5.6 Conclusions

DNA taxonomy can help match individuals of different sexes that belong to undescribed species, assign species names to undescribed genders, and facilitate the discovery of new species, and DNA barcoding have the potential to help identify specimens successfully. Without the correct identification and classification of individuals into their respective taxa, the basic data that most ecological studies rely on are flawed, and therefore the conclusions arising from them may be incorrect. Molecular information obtained through such techniques can be crucial in providing and completing information used in ecological studies, such as community ecology (chapter 4) or assessments of the effects of human disturbances on native and exotic organisms (chapter 5).

In this study, molecular data have been used to match specimens of different genders (*C. blesti*) and avoid misidentification of specimens that could have been considered exotic (genus *Hypoblemum*). Phylogenetic analyses confirmed the distinction and classification of specimens that showed ambiguous morphological characters into separate entities (genus *Laestrygones*), allowing their correct quantification in subsequent ecological analyses (see chapter 4).

In addition, an expected complex phylogenetic relationship between specimens of two congeneric species (*O. orophila* and *O. poppelwelli*) has been revealed, showing the potential of DNA taxonomy as a tool to recognise taxa that may require further taxonomic work. This study has provided genetic data on indicator species (*A. hilaris* and *A. flavescens*) that could be useful in future DNA based methods that identify and quantify the number of specimens at unidentifiable stages that otherwise would not be considered. Juvenile individuals could then be included in studies on community ecology, leading to a more complete view of spiders assemblages and a deeper understanding of spiders' functions in ecosystems.

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Chapter 4

Patterns in spider assemblages in response to plant structure

4.1 Abstract

The present study analysed and identified the biotic and abiotic factors that drive spider diversity in tussock-covered areas, giving special attention to the families and species with potential as indicators of ecological change. This study was conducted in Te Papanui Conservation Park, Central Otago and sites were set up in a nested design with each site divided into five plots. Spiders were collected through turf sampling and pitfall trapping in the summers of 2007/08 and 2008/09. Explanatory variables that explained spatial variation in spider assemblages were identified. Variables were related to environmental gradients between areas with different types of vegetation that indicated differing soil conditions and three dimensional structures of plants. Gradients in botanical composition were also identified through data reduction, which identified distinct plant communities within the ecosystem. Gradients in plant structure and in composition were matched by gradients in spider communities, with families, like Orsolobidae, favouring areas with marshland vegetation, and aerial web building families, such as Linyphiidae, preferring sparsely distributed patches of shrubby plant species. Following confirmation of relationships by individual variables, it was concluded that environmental factors, such as soil moisture, affect the plant composition and structure in tussock ecosystems, which in turn determine the spider community composition and relative abundances of families and species. This information allows the

identification of certain families and species, such as Lycosidae and *Anoteropsis hilaris* and *Anoteropsis flavescens*, respectively, as potential indicators of the tussock cover, and therefore the physical structure and habitat availability in a tussock area, suggesting that these two species could be included in monitoring programs for conservation management.

Keywords— Physical structure, botanical composition, vegetation gradient, spider assemblage, tussock grasslands, indicator species.

4.2 Introduction

Conservation planning and management requires objective scientific knowledge and information on the areas or ecosystems under protection or restoration. This includes an understanding of ecological processes and interactions between the elements and organisms that play a role within the systems. For instance, restoration programs that aim to return to pre-human conditions require understanding the changes in interactions between biotic and abiotic factors that have been altered.

As a major element of global biodiversity (Wilson, 1987), arthropods provide fundamental functions in both natural and modified ecosystems, hence they are increasingly used for nature conservation management (Schmidt et al., 2008). However, there is still an immense lack of information on the major arthropod taxa and their role in ecological processes, particularly in regions classified as hotspots of biodiversity at a global scale, such as New Zealand (Myers et al., 2000).

Native tussock grasslands of New Zealand have been the subject of much ecological research, mainly focused on conservation values, and ecological, economic and ecosystem functions of their botanical components (Brockerhoff et al., 2008; Bulloch, 1973; Jensen et al., 1997; Mark, 1969; Mark and Dickinson, 2008; Mark et al., 2009). Despite recent work on tussock grassland areas, invertebrate biodiversity patterns and their response to human modification and disturbance are still poorly known (Barratt et al., 2009, 2005). Furthermore, with a few exceptions (Hay et al., 2008; Kelly et al., 2008; White and Sedcole, 1993), there is even more limited information on the associations and interactions of invertebrates with their physical environment or the botanical

component of native grasslands.

Spiders (Araneae) play a major role in most terrestrial ecosystems (Wise, 1993) due to their generalist predatory habits. Spiders have a significant effect on invertebrate herbivore pests (Sunderland, 1999), their wide range of predatory behaviours allows them to occupy a variety of niches (Wise, 1993), and their distribution and assemblages are influenced by environmental conditions (Ziesche and Roth, 2008) and natural or human disturbances (Buddle et al., 2000; Clausen, 1986; Doran et al., 1999). These characteristics, along with their ubiquity and ease of collection make them appropriate indicators of ecological changes (Churchill, 1997), land management (Downie et al., 1999; Gibson et al., 1992), prey availability, habitat quality and heavy metal pollution (Marc et al., 1999).

In order to understand the response of spiders to variation in their environment it is necessary to distinguish the relationships between their assemblages and biotic and abiotic factors. Physical features of the vegetation influence spider assemblages by characterising the three dimensional space in which they live - whether they build webs or hunt actively on the ground. In addition, botanical composition of the vegetation may also determine spider assemblages by affecting herbivore prey associated with specific plant species (plant-host association) (Dennis et al., 2001). Such associations may be observed, for instance, in species of the family Thomisidae, which often ambush their prey by mimicking the coloration of the flowers that they sit on. The success of their camoflage depends on matching the colouration of the spider with that of the flower of a particular plant species or taxon. The association between vegetation and spider community in a given ecosystem has been investigated by looking at the effects of physical structure of the vegetation and litter, prey availability and microclimatic conditions, among other factors (see Uetz, 1991 for a review). Furthermore, some studies have tested the habitat heterogeneity hypothesis, which predicts an asymptotic increase in arthropod abundance and species richness with greater plant structural heterogeneity and species richness (Dennis et al., 1998; Jimenez-Valverde and Lobo, 2007).

Plant architecture or physical complexity can determine spider species diversity, either through spatial requirements of web spiders (Greenstone, 1984; Marc et al., 1999) or other functions, such as protection from vertebrate preda-

tors (Gunnarsson, 1990). Structurally more diverse vegetation can also create more available habitats, thus providing opportunities for species or families with different niche requirements that correspond to different foraging techniques (Dennis et al., 1998; Greenstone, 1984). Also, amount of litter, and composition and cover of the vegetation at ground level can have a significant influence on spider assemblages by creating shelter from predators and inclement weather conditions (Schmidt et al., 2008; Ziesche and Roth, 2008). On the other hand, spider communities can enhance plant diversity by exerting a control over invertebrate herbivores (Schmitz, 2003).

This is the first study that looks explicitly at the diversity patterns of spider assemblages in native New Zealand tussock grasslands in relation to the physical characteristics and composition of the vegetation. The objective of this study is threefold: (1) to contribute to the poorly known arachnofauna of native tussock grasslands of New Zealand through intensive sampling; (2) to analyse the characteristics of plant communities in tussock grasslands that drive or explain diversity of spider assemblages; and (3) to test the hypothesis that spider diversity is a function of plant diversity, and tussock cover and density.

4.3 Methods

Sampling was conducted within Te Papanui Conservation Park (45°40'S 169°45'E), on the Lammermoor and Lammerlaw Ranges in Central Otago, South Island, New Zealand (Figure 4.1). This 21,000-ha park was created in 2003 when, following pastoral lease tenure review, lands that had been purchased by the Nature Heritage Fund were added to previously existing protected reserves. Te Papanui Conservation Park is one of the largest protected areas of native tussock grasslands in New Zealand, and it is regarded as an area of high ecological and landscape value as well as economically important because of its water catchment function for the city of Dunedin (Department of Conservation, 2009). Since the 1997 Tenure Review, livestock have not been allowed in the area included in the park. As a result, the area has only been exposed to grazing by feral animals, such as red deer and pigs.

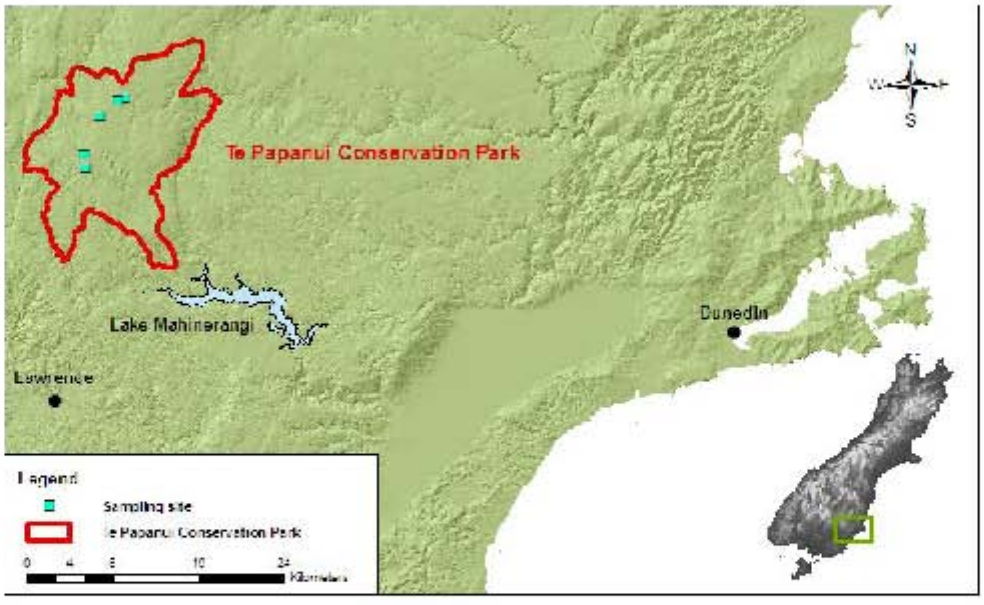

Figure 4.1: Te Papanui Conservation Park, Central Otago

Altitude in the park ranges between 420–1150 m and the mean annual temperature and rainfall vary between around 4–8°C and 1000–2000 mm (NIWA, National Institute of Water and Atmospheric Research, 2003a,b) respectively, both varying with altitude. Most of the ranges are part of an alpine plateau dominated by narrow-leaved snow tussock grass (*Chionochloa rigida*), although shrubby (Figure 4.2) and wetland vegetation is also present in scattered patches around alpine ponds (Figure 4.3).

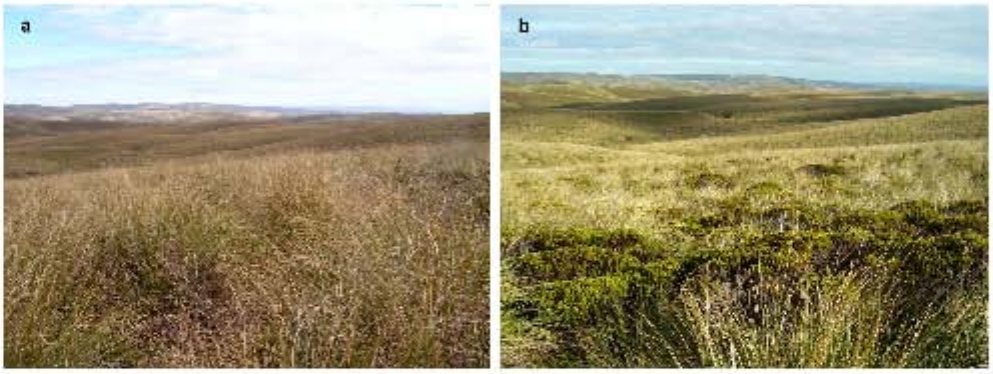

Figure 4.2: Vegetation in Te Papanui Conservation park.(a) Tussock grasses,(b) Shrubby vegetation in foreground.

Figure 4.3: Vegetation in Te Papanui Conservation park. Vegetation around highland tussock grassland ponds.

4.3.1 Sample collection

A hierarchical or nested design of five sites with five circular plots each was selected for this study (Figure 4.4). Sites were randomly located within 500 m from each side of the mountain track that runs through the centre of the park in order to minimise time taken for sample collection and transport to the vehicle. Plots were set up in the shape of an 'X' within each site, with one plot in each corner and one in the centre of an imaginary square. Within each site the distances between the corner plots and the centre plot, and between the corner plots were 50 m and 70.71 m, respectively. Each plot had an area of 400 m^2 (radius of 11.28 m).

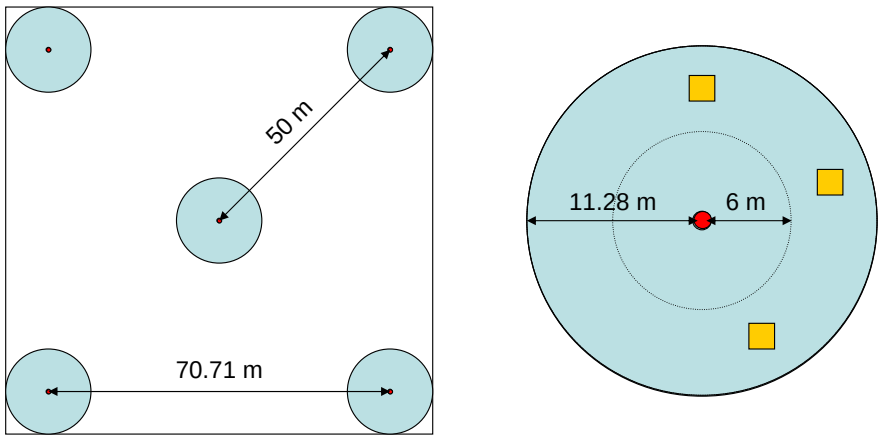

Figure 4.4: Sampling design. Site with five plots and close-up of plot with inner 6 m circle. Red dots represent pitfall traps and orange squares turf samples.

Spiders were collected by turf extraction and pitfall trapping, which have been shown to be effective in tussock grasslands (see chapter 2). Turf samples were collected from the outer area of each plot avoiding an inner circle of six metres radius in order to prevent disturbance or effects on the capture of pitfall traps, which were placed in the centre of each plot (Figure 4.4). One pitfall trap was set up per each plot, giving five traps per site and a total of 25 pitfall traps. These two sampling methods were combined in order to obtain a more complete picture of spider assemblages; turf samples provided information about the density of spiders whereas pitfall traps targeted ground active and nocturnal species that may not have been captured through turf sampling. Three turf samples were collected and pitfall traps replaced from each plot every two weeks during the summer of 2007/2008 between December and March in order to cover spiders' most active period. Three additional turf samplings were conducted in December 2008, and January and February 2009.

Sorting of specimens was conducted at the facilities of AgResearch, Invermay and identification to species or, when not possible, to morphospecies, at Lincoln University. Specimens were identified using available taxonomic keys (Forster, 1967, 1970; Forster and Blest, 1979; Forster et al., 1988; Forster and Wilton, 1968, 1973; Vink, 2002) and classification followed Platnick Platnick (2010). When encountering specimens of different sex belonging to non-described morphospecies, they were matched by sequencing and analysing the cytochrome *c* oxidase subunit 1 of their mitochondrial DNA (see chapter 3). Subsequent specimens considered as morphologically non-distinguishable from the sequenced specimens were classified as belonging to the same species. Specimens were stored in 95% ethanol and deposited in the Entomology Research Museum, Lincoln University.

4.3.2 Turf sampling

Turf extraction was selected as the main sampling method because of its high yield of arthropods in general and spiders in particular. Despite the great physical effort and time that it requires, turf extraction provides very useful data as it allows estimation of macroinvetebrate density (Barratt et al.,

2005, 2006). The sampling unit was 0.1 m^2 and extraction was carried out as described by Barratt et al. (2005). Each sample was collected by placing a 31.7 x 31.7 cm metallic quadrat randomly in the area between the boundary of the inner 6 m circle and the edge of the plot (Figure 4.4) and extracting the turf underneath it to between 5–10 cm of depth using a spade. The amount of soil collected in the sample was minimised as this simplified sample transport and the purpose of the sampling was to collect the top layer of the soil, where the vegetation lies and practically all spiders dwell.

Invertebrates were extracted from the turf samples in modified Tullgren extraction funnels, 40 cm below a 150 W light bulb, at AgResearch Invermay. When funnels were not available, samples were stored in a cold room at 4°C for a maximum of three weeks, until extraction could commence. The extraction time was four days; preliminary experiments indicated that this was sufficient for the extraction of all spiders from turf samples (unpublished data). Mono-propylene glycol was used as preservative because of its DNA preservation properties (Vink et al., 2005), which allows for DNA extraction from the specimens.

4.3.3 Pitfall traps

Each of the 25 pitfall traps consisted of a metallic cylinder and a collection pot of 8 cm diameter and 9 cm depth, which was dug into the ground. The rim of the pot was kept flush with the ground and a square roof placed 3–4 cm above the ground prevented rain and plant debris from entering the trap. Mono-propylene glycol was used as preservative, which prevented the content of the traps from drying out in the field.

4.3.4 Botanical composition

A vegetation survey was conducted in March 2009 and the same metallic 0.1 m^2 quadrats (see above) were used to collect information on the percent cover of each plant species. Each circular plot was divided into three areas according to the distance between their edge and the centre of the plot; (1) up to three, (2) six and (3) 11.28 metres from the centre of the plot. The purpose of this design was to analyse the variation within each plot as well

as to investigate the possible effects that differences between the inner (6 m radius) and outer circles (the remainder of the plot) may have had on the numbers and composition of spiders collected by turf and pitfall samples. Five quadrats were sampled from each of the three areas within each plot, totalling 15 samples per plot.

4.3.5 Statistical analyses

A set of preliminary analyses were conducted to detect any differences in the number of plant species between three areas within plots and sites; analyses of variance (ANOVA) of log(x+1) transformed number of plant species per area, plot and site at both sample and plot level. As no significant differences were found, vegetation sample data from the three different areas were pooled for subsequent analyses, which gave a total number of 15 samples or 1.5 m^2 of sampled area per plot. This area was the sample unit used in all analyses of turf data. As the analyses focused on the plot level, all samples collected from the same plots throughout all sampling dates were pooled separately for turf and pitfall samples and then averaged for an area unit of 1.5 m^2 (15 quadrat samples) and individual pitfall trap, respectively, prior to any analyses. Principal component analysis (PCA) was then carried out for data reduction and to uncover trends or gradients in environmental factors that may explain differences in spider assemblages among plots ($PCA_{physical}$). PCA was selected because of the mostly linear relationships between the analysed variables (Legendre and Legendre, 1998; McCune and Grace, 2002). The variables representing physical and botanical characteristics of the vegetation included in the analysis were basal area of tussocks, mean and maximum height and percent cover of tussocks, percent cover of plant litter, woody plants, lichen and moss, number of plant species and Simpson's D and Shannon's H' diversity indices of plant species. Data were pooled per plot, averaged per area unit and additional geophysical information on the altitude and slope of plots added before the analysis. Correlation coefficients between the coefficient values of the variables and their observed values were used to select the variables that were best explained by the principal components. As the principal components or axes obtained through data reduction represent trends or

gradients in the variables that they explained best, they can be used as composite variables that combine correlated and uncorrelated variables. By doing this, information from such explanatory variables can be retained while avoiding collinearity between them, which can sometimes hinder interpretation and analysis of ecological data (Graham, 2003). The new composite variables can then be used in analyses, such as linear regression models or ANOVA (Beals, 2006; Boyer and Fong, 2005; Ellison et al., 2004; Somershoe and Chandler, 2004; Willis et al., 2005), by using the values of the sites, or plots in the case of this study, on the principal components. This technique allows the description of the community without constraining to a limited number of variables, unlike some ordination methods, such as canonical correspondence analysis (CCA) (McCune and Grace, 2002).

Another PCA was conducted on the percent cover for each plant species in each plot, per area unit ($PCA_{botanical}$). Species with a total cover equal or lower than 0.025 m^2 (0.0667% of the total area or 25% of a single sample) were excluded from the analysis and values of the remaining species were submitted to square root (x+0.5) and Hellinger transformations (Legendre, 2001). This PCA also revealed differences in plots and provided axes based on the values of the principal components that showed gradients in plant composition, which were then used as composite explanatory variables for spider assemblages as with the previous PCA. A set of preliminary analyses of variance (ANOVA) and analyses of similarities (ANOSIM) were also carried out to find out whether the nested design had an effect on the spider assemblages at plot level. One way ANOVAs were used to test the effects of sites on the number of individuals, species and families. All three response variables for the turf data and the number of spider individuals for the pitfall trap data were log transformed prior to analyses. Then, ANOSIMs were used to test the effects of sites on species and family composition, where the values were square root (x+ 0.5) transformed. The three similarity measures calculated for the ANOSIMs were Jaccard's index (a measure of presence/absence), and Euclidean distance and the Steinhaus coefficient S_{17} (also known as Bray-Curtis index), which also take abundance into account. Singletons, doubletons and tripletons were excluded from all ANOVAs and ANOSIMs on species and families assemblages as they were considered as possible vagrants. As the nested design had an affect

on spider assemblages, linear mixed-effects models were constructed for four groups of response variables: (1) number of spider individuals, species and families; (2) number of spiders belonging to four guilds - runners, stalkers, sheet web builders and aerial web builders - based on Uetz et al. (1999); (3) number of individuals per family, and (4) number of individuals per species. Three sets of explanatory variables were selected for the models; (1) the values of the first two principal components obtained in the PCA on the physical and botanical characteristics of the vegetation ($PCA_{physical}$), (2) the values of the first two principal components obtained in the PCA on the plant species composition ($PCA_{botanical}$), and (3) the individual values of all explanatory variables included in the first PCA per plot. The purpose of using the first two PCA components was to analyse the changes in the mentioned response variables in relation to the obtained gradients and thereby obtain a broader view of the patterns of spider assemblages than that provided by individual explanatory variables. The minimal adequate models were selected by forward and back selection of variables by conducting Wald tests and also using Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) (Burnham and Anderson, 2002) as additional selection criteria. All data analyses were carried out using the software R 2.9.0 (R Development Core Team, 2009) and GenStat 12 (Payne et al., 2009) for the linear mixed-effects models.

4.4 Results

A total of 14,465 spider specimens, of which 2,119 were adults belonging to 53 morphospecies from 18 families, were collected from 750 turf samples during the two sampling seasons. The 175 pitfall traps captured 3,634 individuals, of which 1,137 were adults of 33 morphospecies from 15 families. The percentages of adult specimens in turf and pitfall samples were 14.7% and 31.3%, respectively. Pitfall traps collected more spider species than turf samples for any given number of samples, as indicared by the species accumulation curves (Figure 4.5). Lists of spider species, morphospecies and families are shown in Table C.1 of Appendix C.

ANOVAs on the number of spider species, individuals and families per

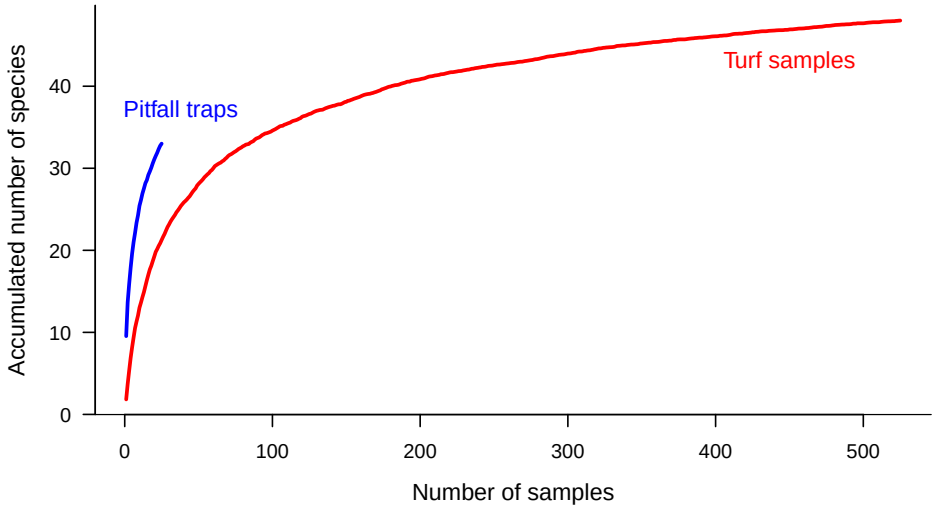

Figure 4.5: Species accumulation curves of turf samples (in red) and pitfall traps (in blue).

turf area unit showed different results, with the five sites differing significantly in the number of individuals ($F_{4,20}$=3.0212, p=0.042) and families ($F_{4,20}$=3.5053, p=0.025) but not in the number of species ($F_{4,20}$=2.1449, p=0.112) (Table 4.1). ANOSIMs on the species and family composition indicated significant differences between sites in Jaccard index, Euclidean distances and Steinhaus coefficient (all p<0.001) (Table 4.2).

Pitfall traps also showed significant differences (ANOVA) in the number of individuals, ($F_{4,20}$=4.836, p=0.007), species ($F_{4,20}$=4.3097, p=0.011) and families ($F_{4,20}$=3.0645, p=0.040) per trap between sites (Table 4.3). ANOSIMs also showed significant differences (p<0.001 for all three indices) in species composition between sites but not for family composition, with only the Steinhaus coefficient (Table 4.4). Therefore, the nested design had an effect on the results, with plots showing more similar spider assemblages to plots from the same site than to plots from other sites. These results suggested that linear mixed-effects models were appropriate for analysing the relationships between the characteristics of the vegetation and spider assemblages.

Pearson correlation coefficients of all explanatory variables included in $\text{PCA}_{physical}$ revealed strong positive correlation between a number of variables related to tussock presence or abundance, such as tussock cover, height

of the vegetation and litter cover, as well as a negative correlation with woody plant cover (Table 4.5). Also, altitude was negatively correlated with the former group of variables and positively with the latter.

Table 4.1: Turf sample data per area unit (1.5 m^2); analyses of variance (ANOVA) of the number of spider individuals, species and families in turf samples among sites. Numbers were calculated per plot and after removing singletons, doubletons and tripletons.

Variable	d.f.	Sum Squares	Mean Squares	F value	Pr(>F)
(a) Number of individuals (log)					
Site	4	0.5568	0.1392	3.0212	0.0422 *
Residuals	20	0.9215	0.0461		
(b) Number of species (log)					
Site	4	0.2314	0.0579	2.1449	0.1127
Residuals	20	0.5395	0.027		
(c) Number of families (log)					
Site	4	0.1954	0.0489	3.5053	0.0253 *
Residuals	20	0.2787	0.0139		

Significance codes: 0.001 '***' 0.01 '**' 0.05 '*'

Table 4.2: Turf sample data per area unit (1.5 m^2); analyses of similarity (ANOSIM) of square root (x+0.5) transformed number of individuals per spider species and family in turf samples among sites. Analysed indices were Jaccard's index, Euclidean distance and Steinhaus coefficient. Numbers were calculated per plot, after removing singletons, doubletons and tripletons and after 1000 permutations.

Variable	ANOSIM statistic R	Significance
(a) Species		
Jaccard's index	0.5798	<0.001 ***
Euclidean distance	0.601	<0.001 ***
Steinhaus coefficient	0.5368	<0.001 ***
(b) Families		
Jaccard's index	0.3794	<0.001 ***
Euclidean distance	0.3907	<0.001 ***
Steinhaus coefficient	0.3269	<0.001 ***

Significance codes: 0.001 '***' 0.01 '**' 0.05 '*'

Table 4.3: Pitfall sample data per trap; linear regression and analyses of variance of the number of spider individuals, species and families among sites. Numbers were calculated per plot and after removing singletons, doubletons and tripletons.

Variable					
Linear Regression	d.f.	r.d.f.	Adjusted R^2	F-statistic	p-value
(a) Number of individuals (log)	4	20	0.39	4.836	0.0068 **
ANOVA		Sum	Mean		
	d.f	Squares	Squares	F value	Pr($>F$)
(b) Number of species					
Site	4	53.44	13.36	4.3097	0.0112 *
Residuals	20	62	3.1		
(c) Number of families					
Site	4	22.8	5.7	3.0645	0.0402 *
Residuals	20	37.2	1.86		

Significance codes: 0.001 '***' 0.01 '**' 0.05 '*'

Table 4.4: Pitfall sample data per trap; analyses of similarity of square root(x+0.5) transformed number of individuals per spider species and family among sites. Analysed indices were Jaccard's index, Euclidean distance and Steinhaus coefficient. Numbers were calculated per plot, after removing singletons, doubletons and tripletons and 1000 permutations.

Variable	ANOSIM statistic R	Significance
(a) Species		
Jaccard's index	0.335	<0.001 ***
Euclidean distance	0.3354	<0.001 ***
Steinhaus coefficient	0.3131	<0.001 ***
(b) Families		
Jaccard's index	-0.02432	0.608
Euclidean distance	0.00616	0.441
Steinhaus coefficient	0.2744	0.002 **

Significance codes: 0.001 '***' 0.01 '**' 0.05 '*'

Table 4.5: Characteristics of the vegetation; Pearson correlation coefficients of physical and botanical characteristics of the vegetation per plot and per sample unit of 1.5 m^2.

Variable	Correlation coefficients														
Tussock cover (m)	1														
Basal area (m^2)	0.83	1													
Mean height (m)	0.69	0.72	1												
Maximum height (m)	0.81	0.75	0.93	1											
Number of tussocks	0.82	0.54	0.55	0.69	1										
Litter cover (%)	0.76	0.6	0.56	0.73	0.62	1									
Woody cover (%)	-0.74	-0.59	-0.53	-0.66	-0.51	-0.93	1								
Moss cover (%)	-0.71	-0.51	-0.41	-0.52	-0.76	-0.57	0.44	1							
Lichen cover (%)	-0.57	-0.53	-0.46	-0.58	-0.55	-0.55	0.35	0.66	1						
Other plant cover (%)	-0.39	-0.23	-0.17	-0.33	-0.47	-0.6	0.47	0.52	0.31	1					
Number of plant species	-0.07	-0.08	0.07	-0.04	-0.07	-0.44	0.43	0.13	0.06	0.63	1				
Shannon's H' (plants)	-0.43	-0.33	-0.23	-0.33	-0.29	-0.63	0.67	0.39	0.14	0.69	0.8	1			
Simpson's D (plants)	-0.44	-0.33	-0.27	-0.36	-0.31	-0.66	0.69	0.41	0.14	0.68	0.74	0.99	1		
Slope (%)	-0.02	0.03	0.1	0.06	-0.02	0.3	-0.2	-0.11	-0.03	-0.19	-0.37	-0.19	-0.21	1	
Altitude (m)	-0.84	-0.54	-0.37	-0.52	-0.7	-0.69	0.75	0.7	0.45	0.32	0.08	0.46	0.48	0.14	1

4.4.1 Data reduction through principal component analysis

Physical and botanical characteristics of vegetation

The first three components of PCA on the physical and botanical characteristics ($PCA_{physical}$) of the plots explained 52%, 18% and 8% of the variance in the data (Table 4.6). Variables related to tussock abundance (tussock cover, vegetation height, litter cover among others) correlated highly with the first component (PC1) negatively, whereas woody plant, moss and lichen cover were correlated positively (Table 4.6, Figure 4.6). The second component (PC2) correlated highly with the number of plant species (0.84), and the Shannon's H' (0.66) and Simpson's D (0.63) diversity indices. The third component (PC3) showed a positive correlation with slope of the plot.

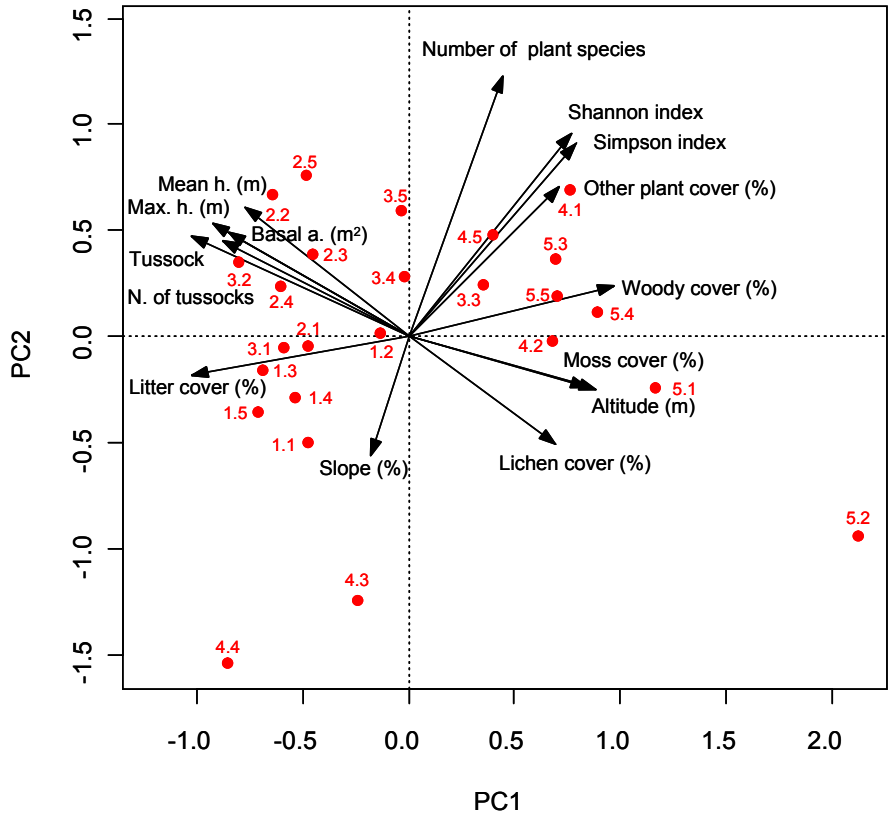

Figure 4.6: $PCA_{physical}$; principal component analysis on physical and botanical characteristics of the vegetation per plot and per area unit of 1.5 m^2. Variables are represented by black arrows and plots by red dots. The first number of the name of each plot indicates the number of the site and the second one the number of the plot within the site.

Table 4.6: Characteristics of the vegetation; single and cumulative explained variance of physical and botanical characteristics of the vegetation per plot and per area unit of 1.5 m^2 by the first six principal components of $PCA_{physical}$.

	PCA component					
	PC1	PC2	PC3	PC4	PC5	PC6
Single variance (%)	0.5198	0.1825	0.0783	0.0668	0.044	0.0331
Cumulative variance (%)	0.5198	0.7023	0.7806	0.8474	0.8914	0.9245

Table 4.7: Characteristics of the vegetation; correlation coefficients between the first four principal components of PCA on the physical and botanical characteristics of the vegetation ($PCA_{physical}$) and the variables included in the analysis. Values of the variables were per plot and per area unit of 1.5 m^2. Largest values are indicated in bold.

	PCA component			
Variable	PC1	PC2	PC3	PC4
Tussock cover	**-0.905**	0.322	-0.077	0.104
Basal area of tussocks	**-0.75**	0.334	0.22	0.165
Mean vegetation height	-0.683	0.418	0.448	0.155
Maximum vegetation height	**-0.819**	0.363	0.307	0.097
Number of tussocks	**-0.779**	0.308	-0.236	-0.213
Litter cover	**-0.911**	-0.123	0.13	0.001
Woody plant cover	**0.859**	0.164	-0.097	-0.288
Moss cover	**0.754**	-0.162	0.305	0.426
Lichen cover	0.619	-0.347	0.032	0.459
Other vegetation cover	0.632	0.484	0.191	0.292
Number of plant species	0.392	**0.835**	-0.059	0.062
Shannon's H' of vegetation	0.683	**0.657**	0.084	-0.193
Simpson's D of vegetation	**0.701**	**0.626**	0.066	-0.195
Slope	-0.159	-0.384	**0.654**	-0.464
Altitude	**0.783**	-0.172	0.413	-0.171

Plant species composition

The PCA on plant composition based on percent cover of plant species ($PCA_{botanical}$) also uncovered certain trends in vegetation. The first three components explained 59%, 11%, 7% of the variance in the vegetation data (Table 4.8), and were highly correlated with a number of species (Table 4.9); PC1 was most highly correlated with *Kelleria dieffenbachii* (0.98) and *Chionochloa rigida* (-0.86) and PC2 with *Coprosma petriei* (0.80) and *Coprosma perpusilla* (0.79) (Figure 4.7). A complete list of plant species is shown in Table C.2 of Appendix C.

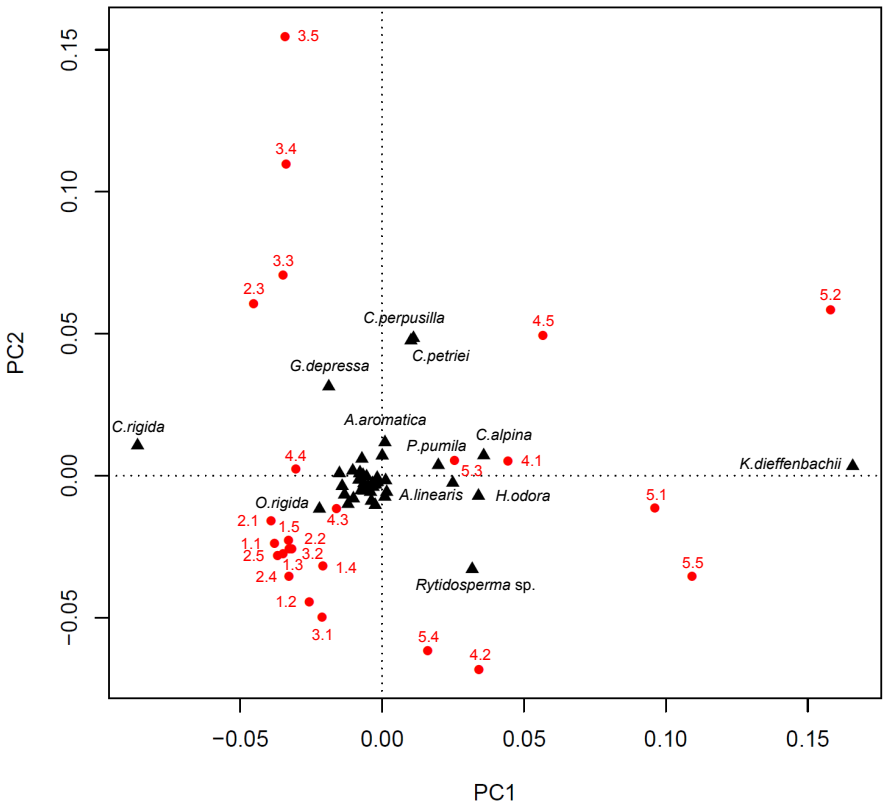

Figure 4.7: Principal component analysis on percent cover of plant species per plot and per area unit of 1.5 m^2. Black triangles represent plant species and red dots plots.

Table 4.8: Botanical diversity; single and cumulative variance explained by the first six principal components of PCA on the abundances of the plant species present in each plot ($PCA_{botanical}$). Species with a cover equal or lower than 0.0667% of the total area were not included in the analysis.

	PCA component					
	PC1	PC2	PC3	PC4	PC5	PC6
Single variance (%)	59.16	11.32	7.5	5.15	4.37	2.44
Cumulative variance (%)	59.16	70.48	77.98	83.13	87.5	89.94

Table 4.9: Botanical diversity; coefficients of correlation between the first four principal components of PCA on the cover of different plant species present in each plot ($PCA_{botanical}$). Greatest values are indicated in bold.

	PCA component			
Species	PC1	PC2	PC3	PC4
Chionochloa rigida	**-0.856**	0.1885	-0.2868	0.1461
Astelia linearis	**0.7097**	0.0227	-0.0756	0.4811
Carpha alpina	**0.8213**	0.2226	0.2860	0.0229
Celmisia sessiliflora	0.2745	0.1397	-0.1506	**0.7323**
Coprosma perpusilla	0.2403	**0.7957**	0.1407	-0.2855
Coprosma petriei	0.2124	**0.8008**	-0.3122	0.1397
Euphrasia sp.	**0.6035**	0.2238	0.4584	-0.1987
Gaultheria depressa	-0.2783	**0.7277**	-0.3574	-0.1280
Gentiana bellidifolia	0.6126	0.1858	0.0078	0.1552
Gentiana grisebachii	-0.2621	**0.6467**	-0.3245	-0.1910
Hebe odora	**0.7489**	-0.0685	-0.3848	0.1314
Kelleria dieffenbachii	**0.9825**	0.0598	0.0549	-0.0214
Leucopogon fraseri	0.0609	-0.0542	-0.0765	**0.6017**
Lycopodium fastigiatum	-0.1327	**0.6430**	-0.2436	-0.2199
Oreobolus pectinatus	**0.6035**	0.2238	0.4584	-0.1987
Pentachondra pumila	0.4101	0.1221	-0.1503	**0.7170**

4.4.2 Effects of gradients in physical characteristics of the vegetation on spiders

A number of response variables appeared to be affected by the first two components of $PCA_{physical}$. Data obtained from turf samples suggested that PC1 had an effect on the number of individuals and PC2 on the number of families, both positive (Table 4.10).

According to turf data, spiders belonging to the guild of ground runners had a positive relationship with PC1 and the number of spiders from the families Gnaphosidae and Mysmenidae showed a positive and negative relationship with PC1 respectively. Turf data also showed that PC2 had a positive effect on the number of Hahniidae (Figure 4.8f) and Lycosidae (Figure 4.8e); the latter was also confirmed by the data from pitfall traps (Table 4.11). Pitfall trap data suggested a negative effect of PC1 on Lycosidae. The effects of the two first components on certain species were confirmed by data from both sampling methods; *Rinawa cantuaria* and *Anoteropsis hilaris* had a positive relationship with PC2 and PC1, respectively, and *Anoteropsis flavescens* decreased with an increase in the value of PC1. The full list of the effects of the two first components of $PCA_{physical}$ on families and species are shown in Table 4.10 (turf data) and Table 4.11 (pitfall trap data).

Table 4.10: Turf spider data per area unit (1.5 m^2); effects of the first two principal components of PCA on the physical and botanical characteristics of the vegetation ($PCA_{physical}$) on the number of spider individuals, species, families, and the number of individuals of each family, guild and species obtained through linear mixed-effects models. Only results from the selected best fitting models are shown.

Response variable	Explanatory variable	Average effect	Std. error	Wald statistic	n.d.f.	*F* statistic	d.d.f.	*F* pr
(a) Spider diversity								
Number of individuals log(x)	PC1	0.159	0.065	5.95	1	5.95	21.6	0.023
Number of species log(x+1)	PC2	0.109	0.032	11.96	1	11.96	22.7	0.002
Number of families log(x+1)	PC2	0.084	0.021	15.42	1	15.42	21.3	<0.001
(b) Spider guilds and families								
Runners log(x+1)	PC1	0.289	0.107	7.33	1	7.33	9	0.024
Gnaphosidae sqrt(x+0.5)	PC1	0.143	0.045	10.23	1	10.23	19.3	0.005
Mysmenidae log(x+1)	PC1	-0.346	0.079	19.26	1	19.26	4	0.012
Hahniidae sqrt(x+0.5)	PC2	0.341	0.135	6.32	1	6.32	22.7	0.019
Lycosidae sqrt(x+0.5)	PC2	0.235	0.05	21.91	1	21.91	21	<0.001
(c) Spider species								
Neoramia matua sqrt(x+0.5)	PC1	0.159	0.04	15.76	1	15.76	8.2	0.004
Laetesia minor log(x+1)	PC1	0.624	0.173	12.95	1	12.95	10.7	0.004
Laetesia trispathulata sqrt(x+0.5)	PC1	-0.286	0.088	10.49	1	10.49	14.4	0.006
Anoteropsis hilaris sqrt(x+0.5)	PC1	0.084	0.032	7.03	1	7.03	5.3	0.043
Mysmenidae sp.1 sqrt(x+0.5)	PC1	-0.081	0.023	11.65	1	11.65	3.8	0.029
Trogloneta sp.1 sqrt(x+0.5)	PC1	-0.263	0.077	16.97	1	16.97	5.3	0.008
Orsolobidae sp.4 sqrt(x+0.5)	PC1	0.26	0.063	9.02	1	9.02	7.4	0.019
Parafroneta minuta sqrt(x+0.5)	PC1	-0.328	0.109	13.59	1	13.59	6.2	0.01
Hypoblemum sp. sqrt(x+0.5)	PC1	0.153	0.042	9.52	1	9.52	5.6	0.024
Anoteropsis flavescens sqrt(x+0.5)	PC1	-0.137	0.045	22.5	1	22.5	18.6	<0.001
	PC2	0.219	0.046	10.39	1	10.39	21.4	0.004
Nauhea tapa sqrt(x+0.5)	PC2	0.108	0.033	15.47	1	15.47	20.4	<0.001
Rinawa cantuaria sqrt(x+0.5)	PC2	0.282	0.072	9.6	1	9.6	21.3	0.005
Diploplecta duplex sqrt(x+0.5)	PC2	0.262	0.084	4.78	1	4.78	20.5	0.04
Metafroneta minima sqrt(x+0.5)	PC2	0.142	0.065	5.95	1	5.95	21.6	0.023

Table 4.11: Pitfall data per trap; effects of the first two principal components of PCA on the physical and botanical characteristics of the vegetation ($PCA_{physical}$) on the number of individuals of each family and species obtained in linear mixed effect models. Only results from the selected best fitting models are shown.

Response variable	Explanatory variable	Average effect	Std. Error	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
(a) Spider families								
Lycosidae log(x+1)	PC1	-0.292	0.066	25.6	1	25.6	2.8	0.017
	PC2	0.3	0.083	12.94	1	12.94	7.7	0.007
(b) Spider species								
Diploplecta duplex sqrt(+0.5)	PC1	0.048	0.013	13.53	1	13.53	6.1	0.01
Anoteropsis hilaris sqrt(x+0.5)	PC1	0.15	0.063	5.74	1	5.74	16.6	0.029
Anoteropsis flavescens log(x+1)	PC1	-0.453	0.061	66.24	1	66.24	3.1	0.004
	PC2	0.268	0.079	11.6	1	11.6	7.5	0.01
Parafroneta minuta sqrt(x+0.5)	PC2	-0.063	0.021	8.98	1	8.98	22.5	0.007
Rinawa cantuaria sqrt(x+0.5)	PC2	0.025	0.01	5.82	1	5.82	21.7	0.025

4.4.3 Effects of gradients in botanical composition on spiders

Models based on turf data using the first two components of $PCA_{botanical}$ indicated that PC1 had an effect on more response variables, including number of spider individuals and families, than PC2 (Table 4.12). Unlike the models built with the first two components of $PCA_{physical}$, where only the guild of ground runners was affected, all four guilds responded positively to either PC1 or PC2 of $PCA_{botanical}$, according to the turf data; PC1 had a positive effect on ground runners, stalkers and aerial web builders, whereas PC2 had on sheet web builders.

Data from turf samples identified a negative effect on Mysmenidae and a positive effect on Linyphiidae (Figure 4.8g) and Orsolobidae by PC1. In addition, orsolobids were positively affected by PC2. Models on data from pitfall traps indicated that numbers of species and families were negatively affected by PC2 and suggested different relationships between the first two components and families and species; such as Cycloctenidae that was negatively, and *A. hilaris* positively, affected by PC2 and *A. flavescens* negatively affected by PC1 (Table 4.13). The positive relationship between the number of Linyphiidae and aerial web builders and PC1 was confirmed by both turf and pitfall samples. PC1 of $PCA_{physical}$ and $PCA_{botanical}$ were negatively correlated with tussock (*C. rigida*) cover and highly correlated (0.88) with each other.

Table 4.12: Turf spider data per area unit (1.5 m^2); effects of the two first principal components of PCA on the plant species cover ($PCA_{botanical}$) on the number of spider individuals, families, and the number of individuals of each family, guild and species obtained through linear mixed-effects models. Only results from the selected best fitting models are shown.

Response variable	Explanatory variable	Average effect	Std. Error	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
(a) Spider diversity								
Number of individuals log(x)	PC1	4.012	0.991	16.4	1	16.4	23	<0.001
Number of families log(x+1)	PC1	-1.437	0.302	22.71	1	22.71	3	0.017
(b) Spider guilds and families								
Aerial web builders log(x+1)	PC1	3.996	1.825	4.8	1	4.8	17.7	0.042
Stalkers sqrt(x+0.5)	PC1	3.535	1.373	6.63	1	6.63	5.2	0.048
Runners log(x+1)	PC1	7.151	1.938	13.62	1	13.62	16.5	0.002
Sheet web builders log(x+1)	PC2	5.663	2.692	4.43	1	4.43	21.1	0.048
Linyphiidae log(x+1)	PC1	4.347	1.766	6.06	1	6.06	19	0.024
Mysmenidae log(x+1)	PC1	-5.684	1.454	15.27	1	15.27	4.2	0.016
Salticidae sqrt(x+0.5)	PC1	3.535	1.373	6.63	1	6.63	5.2	0.048
Orsolobidae log(x+1)	PC1	11.617	3.409	13.99	1	13.99	20.9	0.001
	PC2	5.105	2.383	4.59	1	4.59	19.7	0.045
Huttoniidae sqrt(x+0.5)	PC2	-3.546	1.62	4.79	1	4.79	22.7	0.039
(c) Spider species								
Neoramia matua sqrt(x+0.5)	PC1	2.568	0.674	14.51	1	14.51	8.1	0.005
Hypoblemum sp. sqrt(x+0.5)	PC1	2.773	0.72	14.83	1	14.83	7.2	0.006
Mysmenidae sp.1 sqrt(x+0.5)	PC1	-1.319	0.298	19.58	1	19.58	3.6	0.015
Trogloneta sp. sqrt(x+0.5)	PC1	-4.15	1.486	7.8	1	7.8	4.1	0.048
Orsolobidae sp.4 sqrt(x+0.5)	PC1	4.508	0.767	34.57	1	34.57	4	0.004
Laetesia minor log(x+1)	PC1	9.437	2.55	15.23	1	15.23	8.3	0.004
	PC2	6.253	2.143	8.51	1	8.51	22	0.008
Orsolonidae sp.1 log(x+1)	PC1	11.589	3.623	13.59	1	13.59	21.9	0.001
	PC2	6.717	2.409	7.77	1	7.77	19	0.012
Laetesia trispathulata sqrt(x+0.5)	PC2	-3.073	1.154	7.09	1	7.09	20.1	0.015
Micropholcommatidae sp.2 log(x+1)	PC2	5.225	2.465	4.49	1	4.49	20.2	0.047
Huttoniidae sp. sqrt(x+0.5)	PC2	-3.546	1.62	4.79	1	4.79	22.7	0.039

Table 4.13: Pitfall data per trap; effects of the two first principal components of PCA on the plant species cover ($PCA_{botanical}$) on the number of spider species, families, and the number of individuals of each family, guild and species obtained in linear mixed effect models. Only results from the selected best fitting models are shown.

Response variable	Explanatory variable	Average effect	Std. Error	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
(a) Spider diversity								
Number of species sqrt(x+0.5)	PC2	-0.95	0.407	5.44	1	5.44	22.2	0.029
Number of families sqrt(x+0.5)	PC2	-0.865	0.337	6.56	1	6.56	23	0.017
(b) Spider guilds and families								
Aerial webs sqrt(x+0.5)	PC1	2.617	1.005	6.78	1	6.78	11.7	0.023
Linyphiidae sqrt(x+0.5)	PC1	2.727	1.001	7.42	1	7.42	12.4	0.018
Cycloctenidae sqrt(x+0.5)	PC2	-1.636	0.536	9.32	1	9.32	20.8	0.006
(c) Spider species								
Diploplecta duplex sqrt(x+0.5)	PC1	1.036	0.205	25.56	1	25.56	6.3	0.002
Parafroneta minuta sqrt(x+0.5)	PC1	1.128	0.177	40.62	1	40.62	3.4	0.005
Anoteropsis flavescens log(x+1)	PC1	-8.827	0.667	175.04	1	175.04	3.1	<0.001
Toxopsiella lawrencei sqrt(x+0.5)	PC2	-1.636	0.536	9.32	1	9.32	20.8	0.006
Anoteropsis hilaris sqrt(x+0.5)	PC2	2.076	0.813	6.52	1	6.52	22.6	0.018

4.4.4 Effects of individual physical characteristics of the vegetation on spiders

Among all the physical and botanical variables previously included in $PCA_{physical}$, the ones that appeared to have the greatest effect on the response variables when selected individually were tussock cover, woody plant cover and the Simpson's D (Table 4.14, Table 4.15 and Figure 4.8). Selected models based on turf data suggested an increase in the number of spider individuals, species (Figure 4.8b) and families (Figure 4.8d) with greater plant species diversity. Turf data also showed a positive effect of woody plant cover on the number of individuals and of tussock cover on the number of species and families (Table 4.14 and Figure 4.8a and Figure 4.8c). The number of Linyphiidae, Orsolobidae and Salticidae collected through turf samples increased and Mysmenidae decreased with woody plant cover. According to the turf data, height of the vegetation, a variable related to tussock abundance, had a negative effect on the number of Agelenidae and tussock cover had a positive effect on Lycosidae (Figure 4.8h), which was confirmed by data from pitfall samples (Table 4.15).

Turf data showed that Gnaphosidae and Lycosidae (Figure 4.8i) had a positive relationship with Simpson's D index of plant species diversity. The number of species in turf samples showed an increase with tussock cover and Simpson's D index. Only the species *Trogloneta* sp. and *Parafroneta minuta* showed a decrease as the value of Simpson's D index increased. Although the negative response of *P. minuta* to plant diversity was backed by pitfall data, data from the two sampling methods differed in this species' response to tussock cover, with turf data indicating an increase and pitfall data a decrease in the number of individuals (Table 4.14 and Table 4.15).

4.4.5 Overall effects on spider species and families

When modelling with turf data, the same number of models identified positive and negative effects of the first principal component of $PCA_{physical}$ on species, whereas models including the second component only showed positive effects (Figure 4.11). Turf-based models on the gradients represented by the first and second components of $PCA_{botanical}$ had more positive effects on

species than negative effects. Overall, considering turf and pitfall data, physical characteristics of the vegetation, such as tussock and woody plant cover, and Simpson's D index of plant species had effects on a similar number of families and species (Figure 4.11).

Table 4.14: Turf spider data per area unit (1.5 m^2); effects of the physical and botanical characteristics of the vegetation on the number of spider individuals, species, families, and the number of individuals of each family, guild and species obtained in linear mixed effect models. Only explanatory variables from the selected best fitting models are shown. Values of F pr indicate change in deviance when variables are dropped from the full model.

Response variable	Explanatory variable	Average effect	Std. error	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
(a) Spider diversity								
Number of individuals log(x)	Woody plant cover (%)	0.603	0.251	5.77	1	5.77	22.6	0.025
	Number of plant species	0.037	0.012	9.65	1	9.65	19.9	0.006
Number of species log(x+1)	Tussock cover (%)	0.9123	0.2078	19.28	1	19.28	3.3	0.018
	Simpson's D	0.58	0.206	7.91	1	7.91	21.1	0.01
Number of families log(x+1)	Tussock cover (%)	0.8813	0.1255	49.29	1	49.29	3.2	0.005
	Simpson's D	0.44	0.143	9.48	1	9.48	20.4	0.006
(b) Spider guilds and families								
Aerial web builders log(x+1)	Woody plant cover (%)	0.895	0.411	4.74	1	4.74	19.8	0.042
Sheet web builders log(x+1)	Mean height (m)	-7.774	2.442	10.13	1	10.13	20.3	0.005
Stalkers sqrt(x+0.5)	Woody plant cover (%)	1.047	0.3054	11.76	1	11.76	5.6	0.016
Runners log(x+1)	Mean height (m)	-3.393	1.3238	5.46	1	5.46	15.2	0.034
	Number of plant species	0.08	0.024	11.71	1	11.71	21.9	0.002
Agelenidae sqrt(x+0.5)	Mean height (m)	-5.644	2.025	7.76	1	7.76	22.8	0.011
Gnaphosidae sqrt(x+0.5)	Simpson's D	0.794	0.194	16.74	1	16.74	22.7	<0.001
Linyphiidae(log(x+1)	Woody plant cover (%)	1.208	0.374	10.43	1	10.43	21.7	0.004
Lycosidae log(x+1)	Tussock cover (%)	2.46	0.3871	22.64	1	22.64	4.2	0.008
	Simpson's D	1.402	0.327	18.39	1	18.39	11.7	0.001
Mysmenidae log(x+1)	Woody plant cover (%)	-1.674	0.2402	48.57	1	48.57	3.6	0.003
Orsolobidae log(x+1)	Woody plant cover (%)	2.686	0.803	11.2	1	11.2	22.2	0.003
Salticidae sqrt(x+0.5)	Woody plant cover (%)	1.047	0.3054	11.76	1	11.76	5.6	0.016

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Table 4.14 – continued from previous page

Response variable	Explanatory variable	Average effect	Std. error	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
(c) Spider species								
Mahura rufula sqrt(x+0.5)	Mean height (m)	-2.878	1.333	4.66	1	4.66	22.7	0.042
Neoramia matua sqrt(x+0.5)	Tussock cover (%)	-1.47	0.449	10.71	1	10.71	8.6	0.01
Mamoea Rufa sqrt(x+0.5)	Tussock basal area (m^2)	-1.787	0.6571	7.4	1	7.4	10.4	0.021
Nauhea tapa sqrt(x+0.5)	Simpson's D	0.826	0.179	21.24	1	21.24	21.6	<0.001
Rinawa cantuaria sqrt(x+0.5)	Simpson's D	1.6	0.472	11.51	1	11.51	22	0.003
Diploplecta duplex sqrt(x+0.5)	Simpson's D	1.375	0.558	6.06	1	6.06	22.3	0.022
Laetesia minor log(x+1)	Woody plant cover (%)	3.682	0.621	35.2	1	35.2	23	<0.001
	Simpson's D	2.356	0.9599	6.02	1	6.02	18.2	0.024
Laetesia trispathulata sqrt(x+0.5)	Woody plant cover (%)	-0.981	0.374	6.89	1	6.89	20.4	0.016
Metafroneta minima sqrt(x+0.5)	Simpson's D	1.058	0.391	7.32	1	7.32	21.5	0.013
Parafroneta minuta sqrt(x+0.5)	Tussock cover (%)	2.562	0.8586	8.9	1	8.9	4.1	0.039
	Simpson's D	-1.783	0.669	7.11	1	7.11	22.4	0.014
Anoteropsis flavescens sqrt(x+0.5)	Tussock cover (%)	2.681	0.51	15.78	1	15.78	6.6	0.006
	Simpson's D	1.233	0.308	16.03	1	16.03	22	<0.001
Anoteropsis hilaris sqrt(x+0.5)	Woody plant cover (%)	0.3761	0.1185	10.08	1	10.08	5.5	0.022
	Number of plant species	0.024	0.01	5.64	1	5.64	22.5	0.026
Mysmenidae sp.1 sqrt(x+0.5)	Tussock basal area (m^2)	1.467	0.4239	11.98	1	11.98	9.1	0.007
Trogloneta sp. sqrt(x+0.5)	Woody plant cover (%)	-1.317	0.2582	26.02	1	26.02	3.6	0.009
	Simpson's D	-2.388	0.4375	29.78	1	29.78	5	0.003
Orsolobidae sp.1 log(x+1)	Mean height (m)	-7.365	3.033	5.89	1	5.89	21.8	0.024
Hypoblemum sp. sqrt(x+0.5)	Tussock cover (%)	-1.489	0.436	11.68	1	11.68	5.4	0.017
Theridiidae sp. log(x+1)	Simpson's D	2.614	0.979	7.13	1	7.13	21.7	0.014

Table 4.15: Pitfall data per trap; effects of the physical and botanical characteristics of the vegetation on the number of spider individuals and the number of individuals of each family, guild and species obtained in linear mixed effect models. Only results from the selected best fitting models are shown.

Response variable	Explanatory variable	Average value	Std.Error	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
(a) Spider diversity								
Number of individuals log(x)	Tussock cover (%)	4.2	0.615	46.7	1	46.7	3	0.006
(b) Spider guilds and families								
Aerial webs sqrt(x+0.5)	Tussock cover (%)	-1.54	0.656	5.51	1	5.51	9.6	0.042
Hahniidae sqrt(x+0.5)	Simpson's D	0.223	0.103	4.64	1	4.64	22	0.042
Linyphiidae sqrt(x+0.5)	Tussock cover (%)	-1.568	0.655	5.74	1	5.74	9.9	0.038
Lycosidae log(x+1)	Tussock cover (%)	3.051	0.772	15.62	1	15.62	3.1	0.027
(c) Spider species								
Neoramia matua sqrt(x+0.5)	Tussock cover (%)	-0.095	0.03	10.25	1	10.25	3.7	0.037
Orepukia orophila sqrt(x+0.5)	Mean height (m)	-2.042	0.844	5.86	1	5.86	22.9	0.024
Rinawa cantuaria sqrt(x+0.5)	Simpson's D	0.156	0.062	6.29	1	6.29	22.9	0.02
Diploplecta duplex sqrt(x+0.5)	Tussock cover (%)	-0.443	0.084	27.89	1	27.89	3.6	0.008
Parafroneta minuta sqrt(x+0.5)	Tussock cover (%)	-0.751	0.23	6.22	1	6.22	6.6	0.044
	Simpson's D	-0.331	0.135	6.01	1	6.01	21.9	0.023
Anoteropsis flavescens log(x+1)	Tussock cover (%)	4.462	0.61	53.52	1	53.52	3.2	0.004
Anoteropsis hilaris sqrt(x+0.5)	Mean height (m)	-2.497	0.755	10.93	1	10.93	21.6	0.003

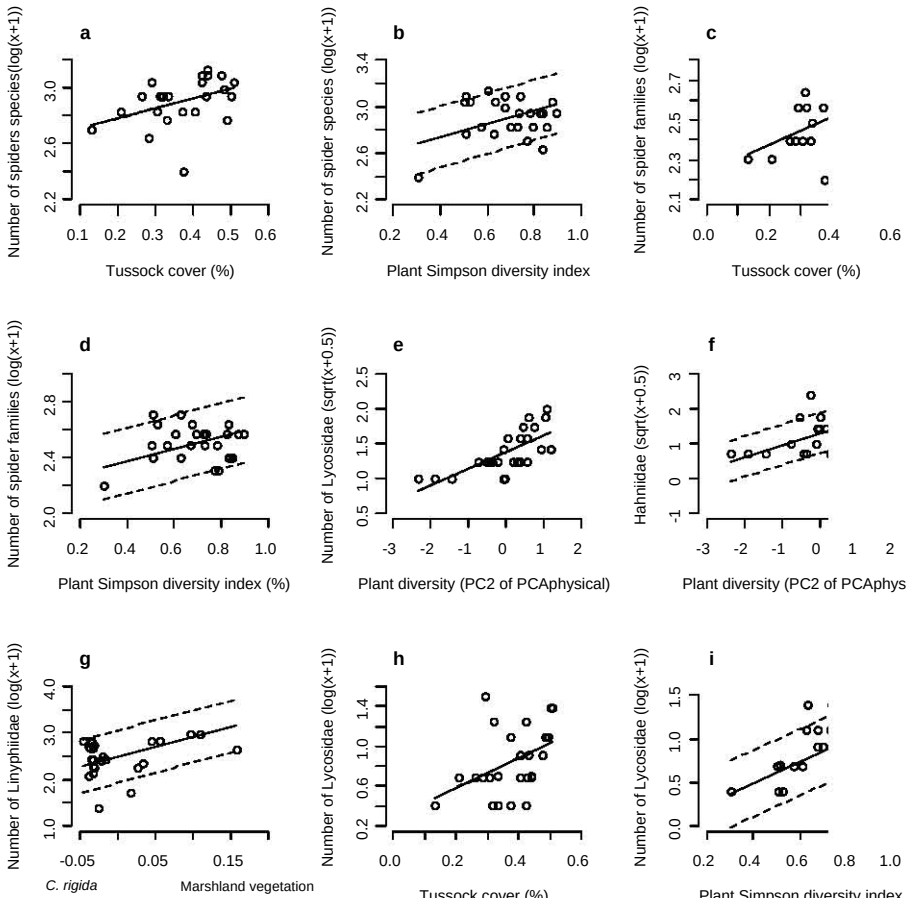

Figure 4.8: Spider response to vegetation characteristics. Linear mixed effect models were based on turf sample data; a) Number of spider species (log(x+1)) as a function of the percent cover of tussocks; b) Number of spider species (log(x+1)) as a function of the Simpson diversity index of plant species; c) Number of spider families (log(x+1)) as a function of the percent cover of tussocks; d) Number of spider families (log(x+1)) as a function of the Simpson's D diversity index of plant species; e) Number of Lycosidae (sqrt(x+0.5)) as a function of the second principal component of PCA on the characteristics of the vegetation ($PCA_{physical}$) (correlated positively with number of plant species; see Table 4.7); f) Number of Hahniidae (sqrt(x+0.5)) as a function of the second principal component of $PCA_{physical}$; g) Number of Linyphiidae (log(x+1)) as a function of the first principal component of PCA on plant species composition ($PCA_{botanical}$) (dominated by species indicating gradient in soil moisture, see Table 4.9); h) Number of Lycosidae (log(x+1)) as a function of the percent cover of tussocks; i) Number of Lycosidae (log(x+1)) as a function of the Simpson's D diversity index of plant species. Solid lines represent estimated average effect of explanatory variables. 95% confidence bands (represented by dotted lines) are indicated in all graphs but they are not distinguishable in some graphs due to their closeness to the line representing the average effect.

4.5 Discussion

4.5.1 Turf sampling vs. pitfall trapping

It appears that pitfall traps are more efficient than turf sampling as the former capture a large percentage (62.3%) of the species collected by the latter with much less sampling effort (Figure 4.5). Also, the percentage of adults in pitfall traps was greater than in turf samples, which means that less time is spent on sorting juvenile specimens that can not provide species data. In addition, pitfall trapping may be a more appealing method as it does not require the physical effort necessary to dig out turf samples and the facilities to extract spiders out of them. These conclusions should be tested through studies designed specifically to compare turf sampling and pitfall trapping.

Depending on the objectives and available resources of project, either pitfall traps or turf sampling may be chosen as the main sampling method. If the aim is to obtain a complete list of species present in an area, pitfall traps may be preferred. On the other hand, if spider density data is required, turf sampling could provide them more accurately as pitfall traps can confound spider activity and trappability with density (see chapter 2). If the human and logistical resources do not allow turf sampling, pitfall traps may be favoured.

4.5.2 Gradients in vegetation

Gradients in the physical structure ($PCA_{physical}$) and botanical composition of the vegetation ($PCA_{botanical}$) in tussock grasslands were found. The former identified a trend in the structure of the vegetation that ranged from areas with a dense tussock cover — which is also reflected by high values of mean vegetation height and large amounts of litter — to areas with a high cover of woody vegetation. The patterns shown by $PCA_{botanical}$ were particularly interesting as the PC1 placed *C. rigida* and *Astelia linearis*, *Carpha alpine*, *Euprasia* sp., *Gentiana bellidifolia*, *Hebe odora*, *Kelleria dieffebachii* and *Oreobolu pecinatus* at opposite ends of a gradient. These species are typical of moist or marshy environments or areas not occupied by tussocks. Therefore, PC1 is most probably reflects a gradient in the drainage or moisture conditions of the soil. On the other hand, PC2 was positively correlated with

Gentiana grisebachii, *Lycopodium fatigiatum*, *Gaultheria depressa*, *Coprosma petriei* and *Coprosma perpusilla*, all of which, except for the first one, have a patchy distribution in alpine tussock grasslands (C. Meurk, pers. comm.), thus revealing another trend in the vegetation.

These gradients confirmed what was observed in the field. The study area in the Lammermoor and Lammerlaw ranges are mostly covered by tussocks with scattered patches of marshland or boggy vegetation on water logged soils, and shrubby or woody vegetation in gullies or areas that tussocks may not have been able to invade. Also, a gradient driven by plant species diversity was found in $PCA_{physical}$ (PC2), which was not correlated with the gradient from tussocks to woody plants.

4.5.3 Spider diversity

Spider assemblages appeared to respond to changes or gradients in tussock vegetation at guild, family and species levels. According to turf samples, the overall number of spiders increased as tussock cover decreased and woody plant cover increased. However, data from pitfall traps pointed to an increase in spider numbers with greater tussock cover (Figure 4.10), which is in line with the idea that tussocks can act as refugia or shelter for invertebrates against unfavourable weather conditions, just as layers of plant litter do (Schmidt et al., 2008). This discrepancy may be explained by the different spider taxa that each sampling method was designed to capture. The differing habitat requirements of these taxa mean that they respond differently to changes in the vegetation. Therefore, the overall number of spiders collected in certain vegetation will change depending on the sampling method and the spider taxa captured. The positive effect of tussock cover on the number of spider species and families indicated by modelling of individual variables (Table 4.14), and $PCA_{botanical}$ in the case of the number of families, suggested that tussock cover may also determine the diversity of spiders. Furthermore, the decrease in the number of species and families detected by pitfall traps as the abundance of woody species with patchy distribution increases seems to suggest that spider diversity may be conditioned by the homogeneity of the tussock cover. The hypothesis that spider diversity is greater in areas with higher tussock cover

or density was supported by the data from turf samples. Spider diversity represented by the number of species and families increased as plant diversity increased along the gradient identified in $PCA_{physical}$ (Figure 4.10). Moreover, modelling of individual variables related to plant diversity confirmed the trend shown by the gradient, with number of individuals, species and families increasing as the number of plant species and values of plant diversity indices increased (Table 4.14).

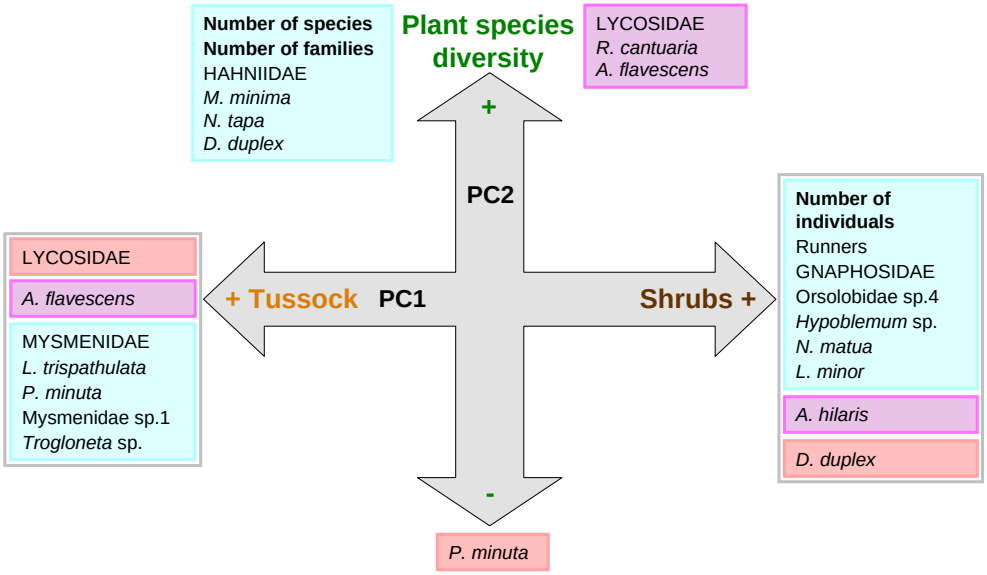

Figure 4.9: Summary of the response of spiders to physical gradients in vegetation. Arrows represent the first (PC1) and second (PC2) principal components of $PCA_{physical}$. Response variables in boxes are variables affected positively ($p<0.05$) by either PC1 or PC2 in the direction of the axis. Blue, red and purple boxes contain variables detected by data from turf samples, pitfall traps and from both methods, respectively. Variables related to spider diversity are in bold, spider families in capital letters, and guilds and species in lower case.

4.5.4 Spider guilds

The lack of knowledge on the biology of many New Zealand spider species hinders their accurate classification into guilds. In addition, certain spider families, such as Desidae and Amphinectidae, can not be assigned single guilds as species within the same family may display different predatory habits. Within the family Amphinectidae, for instance, it is known that species of the genus *Mamoea* prey actively on the ground, whereas the species of the genus *Aoran-*

gia, capture their prey using webs.

Nevertheless, it was possible to classify most of the species collected in this study into guilds, whose abundance patterns shown in the turf data were consistent with what is known about their predatory habits. According to turf data, ground runners were more abundant in plots with greater numbers of plant species and shorter vegetation (Figure 4.10), which represents lower tussock abundance, and thus more open spaces in which to hunt. Turf data also indicated that sheet web building spiders favoured plots with shorted vegetation and greater abundance of sparsely distributed woody plant species (Figure 4.10). Similarly, turf data showed that aerial web spinners preferred areas with greater woody plant presence and lower tussock cover, which was confirmed by pitfall samples. This could be explained by the fact that the more three dimensionally complex architecture of woody plants may fulfil the web attachment requirements that web-spiders depend on (Rypstra et al., 1999) better than tussocks.

4.5.5 Spider families

As plant architecture and density determine spider assemblages (Downie et al., 1995; Gibson et al., 1992), either directly or perhaps indirectly by affecting their prey (Dennis et al., 2001), the presence and abundance of individuals belonging to specific families will depend on the physical characteristics of the vegetation. A number of spider families seem to respond to changes in such characteristics, with a few families displaying particularly interesting patterns. Data from both turf and pitfall samples indicated an increase in the number of spiders from the families Lycosidae as plant species diversity increased. This variable also had an effect on Hahniidae although this was shown only by turf samples. In addition, numbers of Lycosidae showed a strong increase with increasing tussock cover, which was also backed by pitfall data, perhaps because greater tussock cover also means a greater amount of leaf litter, which can create microclimatic and physical shelters for ground active spiders (Rypstra et al., 1999).

Conversely, and according to turf data, two ground active spider families showed different responses to the structure of the vegetation. Orsolobidae

favoured areas with less tussock cover and with scattered woody species or boggy vegetation, whereas Gnaphosidae were also more abundant in areas with fewer tussocks but with greater plant diversity. Neither of these families build webs to capture their prey (Forster and Platnick, 1985; Uetz et al., 1999), therefore it is reasonable to expect they would prefer areas with fewer tussocks and more open spaces. More specifically, these results concur with the observation that Orsolobidae prefer moist habitats with a large moss component (Forster and Platnick, 1985).

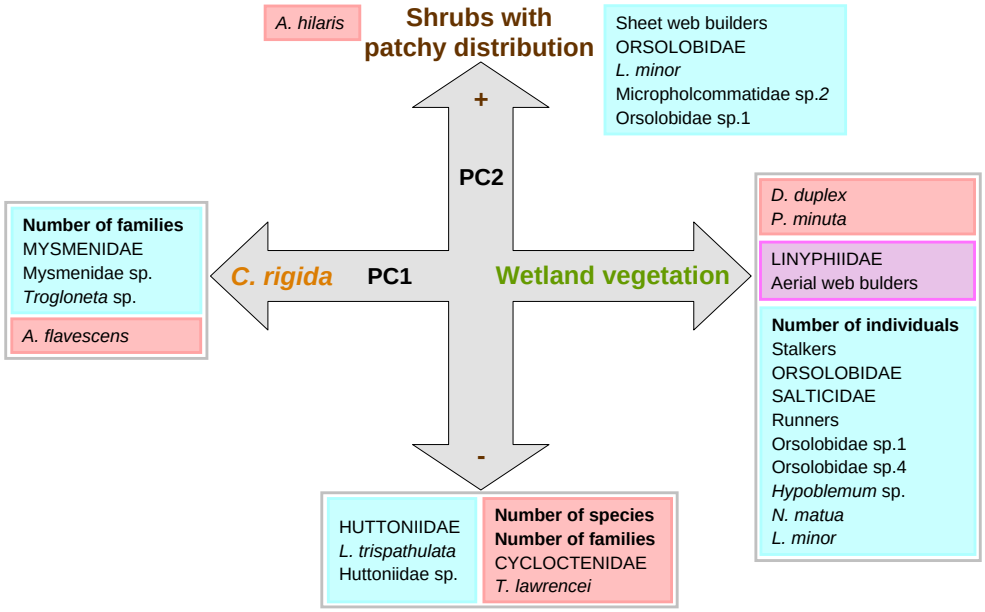

Figure 4.10: Summary of the response of spiders to gradients in the botanical composition of the vegetation. Arrows represent the first (PC1) and second (PC2) principal components of $PCA_{botanical}$. Response variables in boxes are variables affected positively ($p<0.05$) by either PC1 or PC2 in the direction of the axis. Blue, red and purple boxes contain variables detected by data from turf samples, pitfall traps and from both methods, respectively. Variables related to spider diversity are in bold, spider families in capital letters, and guilds and species in lower case.

Turf samples indicated a preference of Linyphiidae for areas with greater cover of woody plants while pitfall samples indicated a preference for areas with less tussock cover. This was consistent with the results of the models based on the $PCA_{botanical}$, which suggested that this family prefers areas with less tussock cover, and was backed by data from both sampling methods. Although Linyphiidae appear to favour areas with a larger presence of tussock forming plants (Cherrett, 1964), they have also been found to differ in their

response to tussock height (Dennis, 2003), which is often correlated with tussock abundance. Thus, the general response of this family may depend on the species present in the area under study and their specific habitat requirements. Nevertheless, given that the species of this family tend to build webs that require multiple attachment points, it is reasonable to expect that these spiders will be abundant in areas covered by woody plants with three-dimensionally complex structures.

It is worth noting that data from turf samples revealed some patterns in Mysmenidae, a poorly known family in New Zealand. Their preference for areas with more tussocks and less woody plants may reflect their need for plants that provide close attachment points for their small webs.

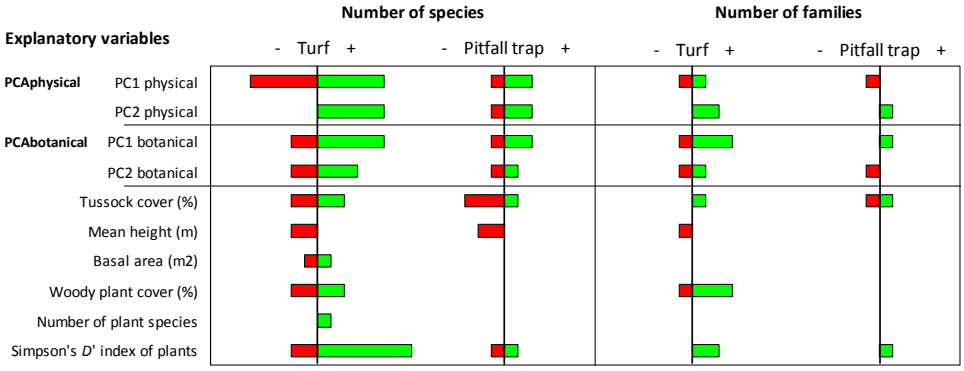

Figure 4.11: Summary of the effects of vegetation on spider species and families. Number of species and families affected by variables representing physical and botanical characteristics of the vegetation (tussock and woody plant percent cover, mean height of vegetation, tussock basal area, number of plant species and Simpson's D diversity index of plant species) and the first two components of $\text{PCA}_{physical}$ and $\text{PCA}_{botanical}$. Only species and families used in the best fitting models based on data from turf and pitfall samples have been included (all modelled species and families are in Table 4.14 and Table 4.15). The length of the bars is equivalent to the number of either species or families affected by each explanatory variable and their colour the sign of the effect (red represents negative and green a positive effect).

4.5.6 Spider species

The general patterns exhibited by certain families in response to specific characteristics of the vegetation can be broken into specific responses of different species. The family Lycosidae was represented by two species with distinct

habitat preferences; *Anoteropsis flavescens* showed a preference for areas with high tussock cover, whereas *A. hilaris* appeared to favour areas with fewer tussocks and more woody plants (Figure 4.12). Although these two congeneric species are known from tussock grasslands (Vink, 2002), *A. flavescens* was previously thought to prefer marshy areas. The findings of this study contradict this, as *A. flavescens* was more abundant in dry areas with greater tussock cover. These results, which were supported by both sample types, indicate clear differences in habitat preferences between the two *Anoteropsis* species and suggest habitat, and perhaps, resource exclusion between them. The discovery of specific habitat preferences highlights the need for more ecological studies focused on native spider species in New Zealand, whose environmental and habitat requirements are still largely unknown.

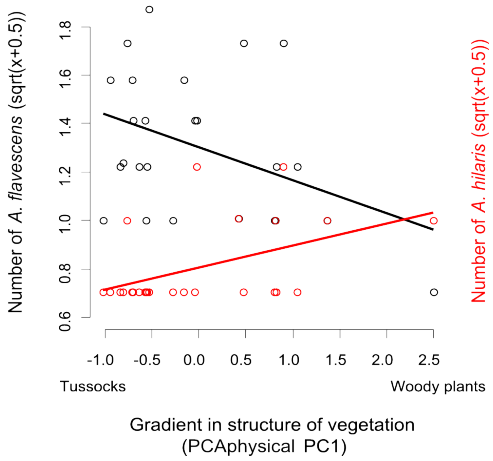

Figure 4.12: Response of two *Anoteropsis* species to physical gradients in vegetation. Response of the number of individuals (squared root + 0.5 transformed) of *A. flavescens* (black line and dots) and *A. hilaris* (red line and dots) to PC1 of $PCA_{physical}$, which represents a gradient from areas covered densely by tussocks to areas with great woody plant cover. Only lines representing average effects are shown.

Another example of differing species requirements is found in the genus *Laetesia*. Data from turf samples suggested that *L. trispathulata* prefers areas with greater tussock and lower woody plant cover, whereas the opposite was true for *L. minor*. *L. trispathulata* and *L. minor* may be another example of resource partitioning by two closely related species although further studies designed specifically to answer such questions are required.

4.5.7 Conclusions

The hypothesis that spider diversity is greater in areas with higher tussock cover or density is largely supported by the results of this study. Although the overall number of individual spiders does not increase with increasing tussock cover or abundance, this may be due to specific patterns or habitat requirements of species that differ in their vegetation preferences, with some thriving in areas with greater tussock cover and others in patches of woody plants. However, there are clear indications that the number of spider species and families increase with tussock cover.

Habitats with diverse vegetation are likely to provide a greater range of resources for herbivorous invertebrates (Crist et al., 2006; Harmon et al., 2003), which in turn can become more abundant and serve as prey for predators, such as spiders. The positive effect of the increase in plant species diversity on the diversity of spiders that was observed may be partly due to such plant-herbivore-predator interactions. Therefore, if it is considered that a greater tussock cover and plant diversity mean greater complexity in the ecosystem, then these results support the contention that a more complex vegetation or environment sustains a greater density and diversity of spiders (Jimenez-Valverde and Lobo, 2007; Rypstra et al., 1999). It has been previously observed that plant diversity has a smaller effect on the diversity of spiders in comparison to measures related to the physical complexity or structure of the vegetation (Dennis, 2003; Dennis et al., 2001). However, the results of this study showed that tussock or woody plant cover had an effect on approximately as many species and families as the Simpson's D index of plant species diversity (Figure 4.11), contradicting at the same time observations suggesting that this diversity index may not be useful when used with plant species to predict the response of spider communities (Beals, 2006).

Diversity patterns differ when investigating each family separately, with Lycosidae and Mysmenidae abundant in areas with higher tussock cover, Orsolobidae and Linyphiidae in scattered areas with fewer tussocks and with either boggy or woody vegetation, and Hahniidae and Gnaphosidae favouring areas with higher botanical diversity. This distinction between taxa can be lowered to species level, where differences in habitat preferences can be seen

between different congeneric species.

4.5.8 Recommendations for monitoring

On the whole, results obtained from turf and pitfall samples agreed, with the only exception being the overall number of spiders. The congruence between the two sampling methods means that both sampling methods may be useful for monitoring spider assemblages in native New Zealand tussock grasslands. However, data from turf samples collected more species and detected more relationships between the number of spiders — total numbers or number of individuals of each species and family — and the measured variables related to the vegetation (Figure 4.11). Therefore, the selection of one method or both for a spider monitoring study should depend on the available resources and the specific aims of the study. This is particularly so given the destructive procedure, great physical effort and time, and equipment necessary to collect turf samples and extract specimens from them.

Another aspect to consider when designing a protocol for monitoring spiders in tussock grasslands is the selection of variables to measure. Gradients in the physical characteristics and botanical composition of the vegetation were reflected in the responses of spider assemblages. The response of the Lycosidae was particularly interesting as it was sensitive to changes in tussock cover and plant diversity according to both turf and pitfall samples. Even within the Lycosidae, there were differing habitat requirements among congeneric species that could potentially used as indicators of habitat availability. For instance, although *A. hilaris* has previously failed as a bioindicator of insecticide contamination (Hodge and Vink, 2000), it may be useful to detect changes in the botanical composition of native grasslands. As *Anoteropsis hilaris* and *Anoteropsis flavescens* are captured in large numbers and their identification is relatively easy, they appear to be appropriate taxa to use as indicators in monitoring programmes that are part of restoration projects in semi-modified native tussock grasslands.

The results of this study have implications for both fundamental questions about plant- arthropod interactions and applied ecology. First, this study supports previous findings about the general effects of the vegetation on spi-

der abundance and composition, which have identified vegetation structure as a major driver of spider diversity. Furthermore, this is the first study that has confirmed such relationships in New Zealand tussock grasslands. Second, the information provided in this study can assist conservation management as it highlights the relevance of the characteristics of tussockland vegetation in determining spider - and most probably other arthropod - diversity. Conservation managers should therefore consider the effects of increasing tussock cover or removal of shrubby vegetation on arthropod diversity when planning programmes aimed at improving or restoring protected areas.

4.6 Acknowledgements

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Chapter 5

Effects of fire on spider assemblages

5.1 Abstract

The effects of controlled fire in semi-modified tussock grasslands were evaluated through an eight-year-long experimental study at Deep Stream, Central Otago, South Island, New Zealand, where changes in spider communities were assessed over a period of seven years, spanning a major fire event in the third year.

A larger project looking at the changes in invertebrate diversity of native tussock grasslands of New Zealand provided the opportunity to study and demonstrate the profound effects of fire in an ecosystem not adapted to regular burning. In this experiment samples were collected before and after a controlled fire in plots representing spring and summer burn treatments as well as unburnt control plots. Fire treatments showed significant differences in spider diversity with respect to the control, with number of families, species and values of other diversity indicators declining drastically after the fire and remaining significantly lower for four years. No significant differences in number of spider individuals, species and families were found between spring and summer burn treatments after the year following fire but there were differences in the spider assemblages over time.

Although the overall trend was for a decrease in the abundance of most spider families, the family Linyphiidae showed a large increase in the years following the fire probably through their efficient dispersal methods and ability to colonise new habitats. An increase in the number of exotic linyphiid species, and particularly the species *Diplocephalus cristatus*, an European species well

established in New Zealand, was a major component of this trend.

The results of this study show the importance of addressing the question of the effects of disturbances like fire on the interactions between native — mostly endemic — and exotic species in New Zealand ecosystems and the dangers that invasive species may pose to native biodiversity.

Keywords— Fire, tussock grasslands, spider diversity, summer fire, spring fire, exotic species.

5.2 Introduction

New Zealand indigenous tussock grasslands constitute one of the iconic landscapes of the country, covering around 40% of the land area (Barratt et al., 2005; Wardle, 1991). Recently, the characteristics of native grasslands have been studied (Mark, 1993) and their ecological and economical values highlighted (Brockerhoff et al., 2008; Mark and Dickinson, 2008; Mark et al., 2009). Since the arrival of Europeans in 1840s, native tussock grasslands have been severely modified and exploited for livestock grazing (Floate, 1992). Consequently, exotic plants and invertebrate species have been introduced (Mark and McLennan, 2005), either intentionally as grass that is more palatable for stock or by themselves, taking advantage of the new conditions. Fortunately, in the past 30 years large areas of tussock grasslands have been formally protected (Grove et al., 2002; Mark et al., 2009) or retired from grazing through the Protected Natural Area Program and the crown pastoral tenure review (Crown Pastoral Land Act 1998), and managed in order to protect and promote native biodiversity. As a result, 17% of conservation lands of New Zealand are now indigenous grasslands (Mark et al., 2009). Fire can be used as a control agent of woody growth (Bragg and Hulbert, 1976) and insect pests (McCullough et al., 1998). Although the use of fire in land management may alter community structure, it can be compatible with insect conservation (Panzer and Schwartz, 2000). Furthermore, its repeated use may increase the diversity of certain arthropods at a regional level (Moretti et al., 2002). Controlled burning has been used to transform or improve tussock grassland pasture since the 19^{th} century (Barratt et al., 2009; Mark, 1994). Fire removes litter, eases the establishment of pasture grass species, and promotes

re-growth of tussocks for livestock (Lowther and Douglas, 1992). A common practice in New Zealand is the use of fire in spring (September-November), when temperatures are lower and vegetation moisture higher than in summer (December-February) (Barratt et al., 2006), so that burn-offs can be better controlled.

Previous studies on the effects of fire in tussocklands have identified changes in plant physiology (Mark, 1965), tissue nutrient concentration (Payton et al., 1986), and micro-arthropod communities (Yeates and Lee, 1997). While it is known that fire damages native biota, facilitates the establishment of exotic plant species, and increases soil erosion (Payton and Pearce, 2009), there is still little information on other effects, which is necessary for the adequate management of both protected and exploited grasslands.

Arthropods are becoming the focus of studies in conservation management due to their contribution to total species richness and their role as pollinators, herbivores, predators and prey for vertebrates (Schmidt et al., 2008). Arthropods are sensitive to changes in the environment caused by disturbances such as fire, which can drive their mortality or affect their diversity and abundance indirectly through changes in the amount of litter, vegetation structure or surface moisture (Blanche et al., 2001; Hartley et al., 2007). In addition, nutrient cycling can be altered by the interactions between invertebrates and fire (McCullough et al., 1998). Although generalisation should be avoided as the effects of fire vary according to taxa, and the characteristics and frequency of fire, the study of such effects on arthropods can provide valuable information for land managers (Blanche et al., 2001; Hartley et al., 2007; Moretti et al., 2002).

Spiders (order Araneae) are considered good bioindicators because of their high diversity and abundance (Churchill, 1997; Skerl and Gillespie, 1999), and their important role as generalist predators in food chains (Clarke and Grant, 1968; Marc et al., 1999; Nyffeler, 2000). Spiders have been used as ecological indicators (Clausen, 1986) as well as indicators of anthropogenic disturbance, such as metal pollution (Clausen, 1986; Marc et al., 1999), habitat fragmentation (Maelfait and Hendrickx, 1998), and land and cave management (Doran et al., 1999; Downie et al., 1999; Gibson et al., 1992). Either single spider species or whole communities may be used to obtain information useful

for land management decisions (Brennan et al., 2006; Doran et al., 1999).

A number of studies have highlighted the potential of spiders as indicators of the effects of fire (Moretti et al., 2002; Neet, 1996), with some showing that spider density and diversity depend on the intensity of fire and time since it last occurred (Koponen, 1988; Moretti et al., 2002; Niwa and Peck, 2002; Urones and Majadas, 2002). There has been little research on spiders despite the common use of fire in grassland management in New Zealand and the knowledge that spiders are one of the most important invertebrate predators in tussock ecosystems (Barratt et al., 2005).

This study builds on previous work by Barratt et al (2009; 2006) on the impacts of controlled fire on tussock macroinvertebrates. The main objective of the project of Barratt et al. is to obtain useful information for the management of native grasslands. The study presented here aims to answer specific questions about the short to medium-term effects of burning on the characteristics of spider communities, such as density, species richness and guild structure as well as their recovery time after fire.

The objectives of this study are: (1) to analyse the effects of fire on tussock grassland spider diversity; (2) to investigate the specific changes in spider assemblages over time after fire; (3) to evaluate the presence and abundance of potentially invasive exotic species before and after fire; (4) to test the hypothesis that summer fire has a more detrimental effect than spring fire because of the higher temperatures reached by the former.

5.3 Methods

The study was conducted at Deep Stream, in Central Otago, New Zealand (Figure 5.1 and Figure 5.2). Deep Stream is a good example of medium altitude tussock grassland. No cultivation or fire has occurred in the area for 30 years (Barratt et al., 2006). Dunedin City Council has used the Deep Stream area as a water reserve, which has allowed the area to remain relatively unmodified. See Table 5.1 for full description of the study site.

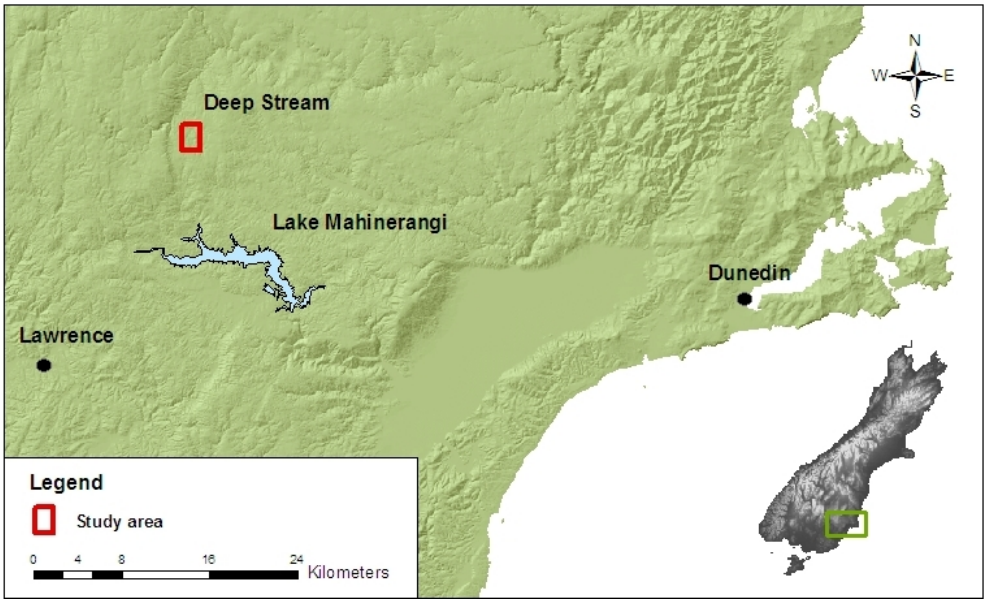

Figure 5.1: Location of the study area.

Burning, sampling and invertebrate extraction are presented here as described in Barratt et al. (2006). The experimental burning occurred in conditions typical of controlled spring fires and accidental summer fires, when conditions are more moist and dry, respectively. Both 2001 spring and 2001 summer fires occurred under the coordination and supervision of Department of Conservation following best practice guidelines. The appropriate timing of fires was determined by calculating a series of indices (detailed in New Zealand Forest Research, 2001) with information provided by the national Rural Fire Authority network of fire stations and the New Zealand Fire Weather Index System (Van Wagner, 1987). Dates of fire events are given in Table 5.1.

Figure 5.2: Study area in Deep Stream.

5.3.1 Experimental design

Three replicates (plots) were set up per fire treatment - spring fire and summer fire - and three more as controls with no fire, totalling nine plots (Figure 5.3). Each plot consisted of a 1 ha square divided into twenty-five 20 x 20 m subplots. The initial purpose of these subplots was to assign them to different research institutions so that a series of studies, looking at the micro and macro-invertebrates and vegetation, could be carried out as part of a larger project on tussock grasslands. As some of these subplots were not used, a subplot per plot was randomly selected every year and used for invertebrate sampling.

Within each plot, the outer subplots were excluded from sampling in order to avoid edge-effects, and turf samples were collected from individual subplots selected randomly from those remaining. Sampling occurred annually in January between the years 1999–2005. There was an additional sampling in March 2001, immediately after the spring fire treatment.

Within a subplot, 20 turf samples were collected between tussocks (intertussock samples) from four rows of five samples each. Nine turf samples that included tussocks (*Chionochloa rigida*) were collected randomly in order to have an approximate representation of the grassland plant cover in the study area. Therefore, a total of 180 intertussock and 81 tussock samples were collected every year. Previous sample locations were avoided during subsequent

Table 5.1: Characteristics of sampling site and dates of controlled fire events.

Tenure [1]	Dunedin City Council water reserve
Altitude (amsl) [2]	640–700 m
Map reference [2]	45°44'S 169°54'E
Dominant vegetation [3]	*Chionochloa rigida*, *Gaultheria nana*, *Poa pratensis*, *Poa colensoi*
Slope and aspect [2]	>10°, predominantly N
Annual rainfall (mm) [4]	993 mm
Mean annual temperature [4]	6.8°C
Soil type [5]	Wehenga silt loam and allophanic brown soils
Soil pH [1]	4.6
Date of spring fire	2-Oct-2001
Soil surface temperature, spring fire [6]	500–1010°C
Date of summer fire	7-Mar-2001
Soil surface temperature, summer fire [6]	300–500°C

[1] Barratt et al. (2006)
[2] Barratt et al. (2009)
[3] Espie and Barratt (2006)
[4] Payton and Pearce (2009)
[5] Hewitt (1992)
[6] New Zealand Forest Research (2001)

collections.

Samples consisted of 0.1 m^2 turf squares (31.7 x 31.7 cm) dug to a depth of approximately 5 cm using a metallic quadrat as a guide. Turf extraction allows calculation of invertebrate density and its comparison between treatments. After transportation of samples to the facilities at Invermay, Dunedin, in individual paper bags (double thickness 'Kleensacks'), they were stored at 4°C for a maximum of three weeks until they were processed (Bremner, 1988).

In the laboratory, invertebrates were extracted from samples by placing them inverted in modified Tullgren extraction funnels, 40 cm below a 150 W light bulb for seven days (Crook et al., 2004). Turf samples were placed in extraction funnels in an arrangement that kept samples from different treatments separated. All the extracted material was stored in 70% ethanol with 10% glycerol at 4°C until sorting.

Samples were sorted by washing them through muslin, which allowed fine silt through but not invertebrates. All spiders were separated from the other invertebrates using a low-power 6.3–40x binocular microscope. Sample collection, and extraction and sorting of invertebrates was conducted by the staff of AgResearch at Invermay, Dunedin. All adult spiders were identified to species or, when not possible, to morphospecies by the author. Only adults were

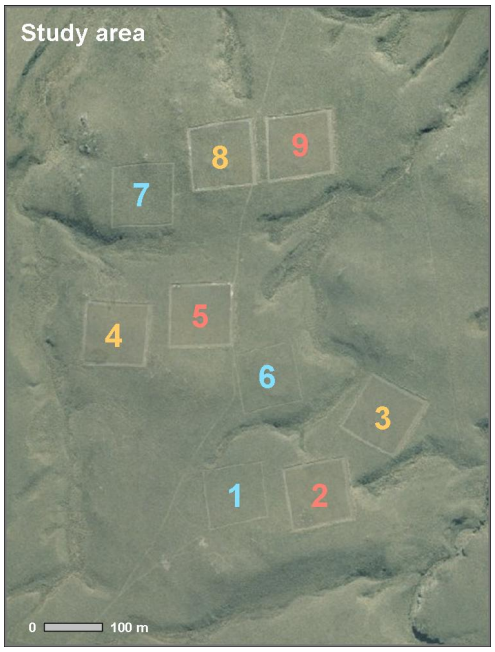

Figure 5.3: Aerial image of study area and layout of sampling plots. Plots 1, 6 and 7 (in blue) were applied the control or unburned treatment, plots 3, 4 and 8 (in orange) spring-burn treatment and plots 2, 5 and 9 (in red) summer-burn treatment.

identified because of the difficulties and uncertainty of juvenile identification (Coddington et al., 1996; Dobyns, 1997). Species and generic identifications were based on available taxonomic literature (Forster, 1967, 1970; Forster and Blest, 1979; Forster et al., 1988; Forster and Wilton, 1968, 1973; Vink, 2002; Vink et al., 2009; Yoshida, 2008) and Platnick's latest nomenclature (Platnick, 2010). A number of specimens of undescribed species were recognised morphologically as conspecific of some of the individuals sequenced previously (chapter 3) and were named accordingly. After identification, all specimens were stored at Invermay in 95% ethanol, for possible future molecular analysis.

5.3.2 Statistical analyses

For comparison, all values of the number of spider individuals, species and families were standardised to 1 m^2 prior to analyses. Numbers for each combination of year, treatment and plot were obtained by calculating the mean values per sample. Singletons, doubletons, tripletons and quadrupletons (species with one, two, three and four individuals, respectively) in all samples were removed from analyses that featured number of individuals per species

or family. These species represented less than 0.1% of all adults. The purpose of this was to remove possible vagrant species, which may not be typical of the area (Coddington et al., 2009).

K-dominance curves were used to assess the differences in spider communities between different years. These curves expose the level of dominance of the community by certain species by plotting the cumulative percentage of individuals per species against their (log transformed) abundance rank (Clarke, 1990). Thus, a lower k-dominance or curve indicates lower species diversity in a community if dominance is considered to have an inverse relationship with equitability, and thus diversity (Lambshead et al., 1983).

Generalised linear mixed effects models (REML) were built in order to test the changes in a number of response variables related to the spider community over time and the effects of the two fire regimes in comparison to the control treatment. The modeled response variables were number of spider individuals, species and families, Shannon's H' species diversity index, percentage of individuals per guild (classified according to Uetz et al., 1999) and percentage of exotic spiders. Other modeled response variables were the species richness estimator ACE (Chao and Lee, 1992) (see chapter 2), number of individuals belonging to families and species that were considered capable of responding to disturbances, and species identified as ecological indicators. Number of individuals, species and families were $\log_{10}$ transformed and Shannon's H' and ACE $\sqrt{(x + 0.5)}$ transformed, whereas all percentage values were transformed through $\sin^2\theta$=(x + 3/8)/(n+3/4) (Pearce, 1965).

Since samples from the same plot were temporally correlated, plot number with year nested within it was set as a random effect. Samples from March 2001 were excluded and only data from samples collected in January every year were included in the models. Thus, the time between each sampling was the same, which allowed application of auto-regressive correlation structure to the models. The predicted values of the response variables were compared through parwise t-tests with p values adjusted with the Bonferroni correction to control for the probability of a Type I error caused by multiple comparisons of the same data. As statistical power decreases with a greater number of comparisons, only a small number of tests were conducted. Pairwise comparisons were selected by inspecting model graphs visually and tested only when differences

between treatments were apparent. Best fitting models were selected based on AIC (Akaike Information Criteria) and BIC (Bayesian Information Criteria), with the models with the lowest index values being preferred.

Differences in spider diversity between different treatments and years were assessed using four dissimilarity measures; Euclidean distance, Jaccard diversity index, and Steinhaus (S_{17}) and Sørensen (S_8) coefficients based on previously transformed data ($\sqrt{(x+0.5)}$). Jaccard index and Sørensen coefficients account for presence/absence of species whereas the Euclidean distance and Steinhaus coefficients also consider abundance (Legendre and Legendre, 1998). Species dissimilarity matrices were used for ordination analyses of spider diversity in different years and treatments. The selected method was Non-metric Multidimensional Scaling (NMDS), an ordination method that reproduces the distances that represent similarity between samples or points in a multidimensional space. In comparison to other types of ordination, NMDS allows the use of biologically meaningful data, such as matrices based on dissimilarity indices of species diversity, preservation of dissimilarity rank order in the distance rank order and identification of a wider range of spatial arrangements among the collected samples (Clarke, 1993; McCune and Grace, 2002).

Two cluster analyses were used to identify similarity in spider diversity between years or treatments; Ward's (1963) minimum variance method and K-means (Legendre and Legendre, 1998). While both aim to minimise the total sum of squares with new partitions, the former is a step-wise hierarchical agglomeration method, whereas the latter groups the points into a predetermined number of clusters whose centroid is closest to the points. Ward's method was applied to dissimilarity matrices based on Steinhaus and Sørensen coefficients. K-means clustering was carried out on the Steinhaus dissimilarity matrix, which had previously been transformed into Euclidean distances though a Principal Coordinate Analysis (PCoA) as suggested by Legendre (Legendre, 2001). The Calinski and Harabasz (1974) pseudo-F-Statistic (C-H) was used as a criterion to select the best clustering.

Species characteristic of each treatment were detected with an indicator species analysis (IndVal), which takes into account the relative abundance and frequency of each species in zones or groups determined by the user. This method has been favoured over other indicator species analyses like TWISPAN

because of its greater identification sensitivity (Dufrêne and Legendre, 1997). IndVal was applied to two species assemblage clusterings or typologies, the ones created by Ward's method on S_{17} and S_8. Indicator values range between 0 and 100, reaching this maximum when a species is present in all the samples of a single treatment or zone. A significance test by a Monte Carlo randomisation procedure (1000 permutations) was carried out for each species and only the significant ones (<0.05) with a IndVal value greater than 25% were considered characteristic of a cluster (Paquin, 2008). All data manipulation and analyses were conducted using the statistical software R 2.10.0 (R Development Core Team, 2009).

5.4 Results

A total of 31,965 spiders, of which 4,564 (14%) were adults, were sorted from 2001 turf samples (seven annual samplings x nine plots x 29 samples + one March sampling x six plots x 29 samples) collected over the eight years of this study. Spring-burnt plots were not sampled in March 2001 as the purpose of these samples was to compare a recently burnt area and an unburnt area. Sixty six species and morphospecies of spiders belonging to 22 families were identified. Approximately 73% of the identified morphospecies are considered native or probably native to New Zealand, 12% are exotic and 5% are of unknown origin (Table D.1 of Appendix D).

5.4.1 Rank-abundance patterns

The analysis of the dominance structure of the spider community through k-dominance curves revealed differences between the two fire treatments and the control plots. While the diversity of the assemblages of control plots was similar over the years (Figure 5.4a), with different years being represented by curves with almost identical shapes, in the summer-burn (Figure 5.4c) and, more particularly, the spring-burn plots (Figure 5.4b), lines representing pre-fire and post-fire years appeared more separated.

It was not possible to visually compare the dominance curves of different years as some of them crossed each other. However, in spring-burn plots there was a clear gap between the curves representing pre-fire and post-fire sampling dates, with the latter ones showing a lower spider diversity (Figure 5.4b). A similar distinction of sampling years determined by fire was observed in summer-burn plots (Figure 5.4c) although in this case the separation was more gradual, with the curves aligned chronologically after the fire.

5.4.2 Generalised linear mixed effect models

According to AIC and BIC and pairwise t-tests, inclusion of year, treatment and the interaction between them as explanatory variables improved all models, and showed significant differences between the control plots and each

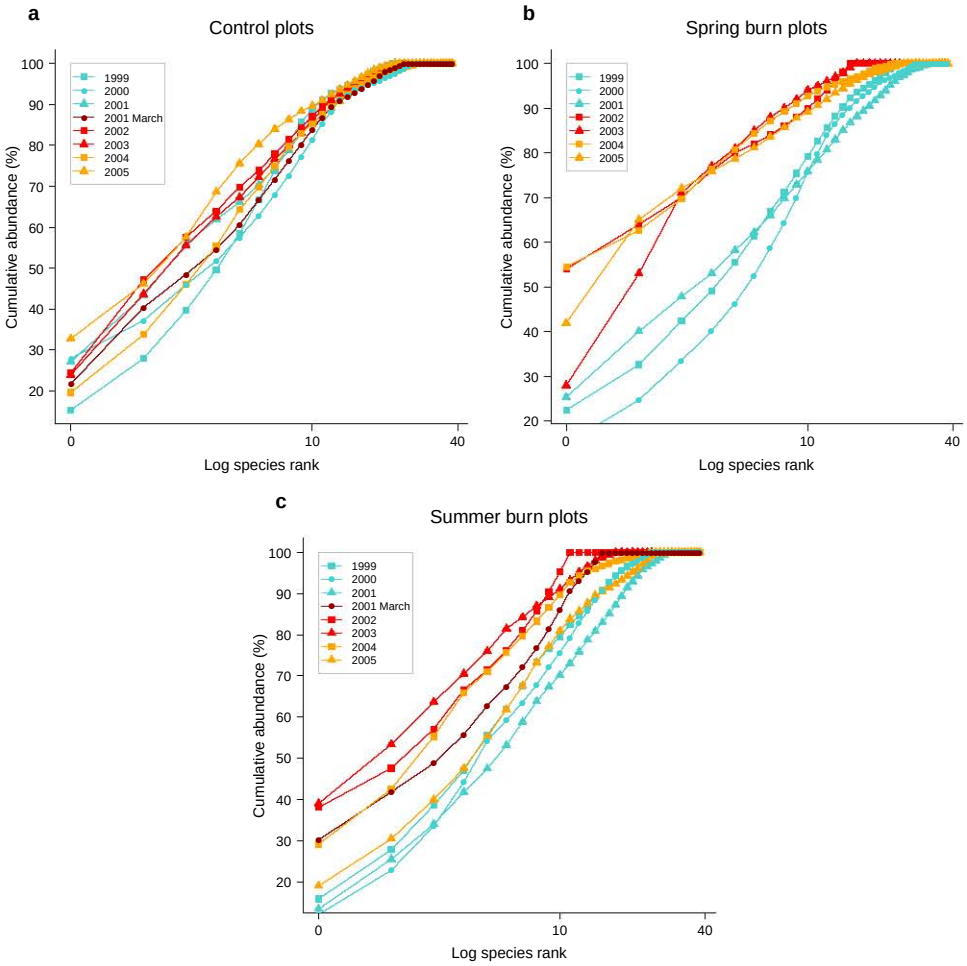

Figure 5.4: K-dominance curves for each sampling year. a. Control plots. b. spring-burn treatment. c. summer-burn treatment. Blue curves represent pre-fire years, dark red curve March 2001, red ones 2002 and 2003, and orange ones 2004 and 2005. Summer fire occurred in March 2001, before sampling, and spring fire in October 2001.

of the two fire treatments in the years following the fire event (Table D.2 of Appendix D).

The $\log_{10}$ transformed number of spider individuals fluctuated in all three treatments over the years (Figure 5.5). There were significant differences between the control plots and the two fire treatments before (2001, $p<0.01$) and after (2002, $p<0.05$) the fire. Spring and summer fire treatments had lower numbers of individuals in the year 2002 and control and spring-burn plots remained significantly different ($p<0.05$) in 2003. The number of species and families ($\log_{10}$ transformed) showed similar patterns, with significant differences ($p<0.01$) between the control plots and the fire treated plots after the

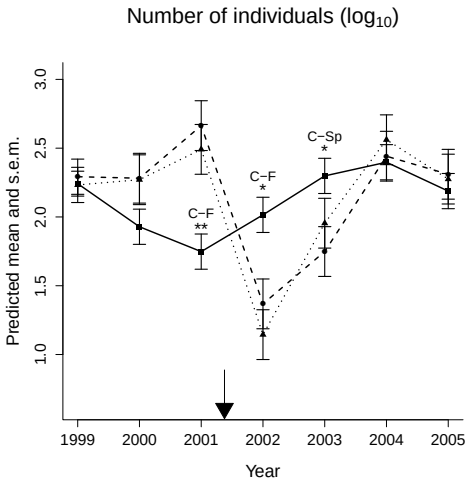

Figure 5.5: Number of spider individuals ($\log_{10}$) per m^2. Predicted means and their standard errors are shown. Solid lines represent numbers in control plots, dashed ones in spring-burn plots and dotted ones in summer-burn plots over the sampling years. Significant differences found through pairwise t-tests are represented by * for $p<0.05$ and by ** for $p<0.01$. Comparisons marked with "C", "F", "Sp" and "Su" indicate differences found between control plots, fire treated plots, spring-burn plots and summer-burn plots, respectively. The arrow indicates the time when the fire occurred.

fire (Figure 5.6a and b). There were also significant ($p<0.01$) differences in the number of species and families between the control plots and the summer ($p<0.05$) and spring ($p<0.01$) fire treatments in the year 2000, respectively (Figure 5.6). Summer fire plots had significantly fewer species than spring plots in the year 2002 ($p<0.05$)

The estimated number of species (ACE) was significantly greater ($p<0.01$) in control plots than in fire treated plots in 2002 and, 2003 (Figure 5.7a). Control plots had a significantly larger ($p<0.01$) value of the Shannon's H' diversity index than summer-burn plots in the year after the fire (Figure 5.7).

The partition of spiders into guilds revealed a different trend in the group of aerial web builders, which increased significantly in treatment plots after the fire (Figure 5.8a). Control plots had significantly lower percentage of aerial web builders in comparison with summer-burn plots ($p<0.01$) in 2003 and both fire treatments in 2004 ($p<0.01$) and 2005 ($p<0.05$), respectively. Although the guilds of ground runners, sheet web builders, ambushers and stalkers were

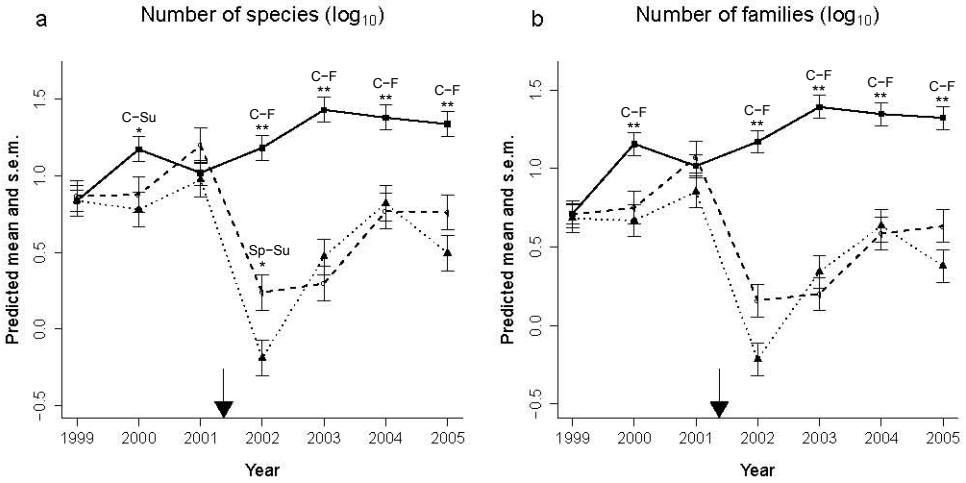

Figure 5.6: Number of spider (a) species and (b) families ($\log_{10}$) per m^2. Predicted means and their standard errors are shown. Solid lines represent numbers in control plots, dashed ones in spring-burn plots and dotted ones in summer-burn plots over the sampling years. Significant differences found through pairwise t-tests are represented by * for $p<0.05$ and by ** for $p<0.01$. Comparisons marked with "C", "F", "Sp" and "Su" indicate differences found between control plots, fire treated plots, spring-burn plots and summer-burn plots, respectively. The arrow indicates when spring and summer fires occurred (both between January 2001 and January 2002).

also modelled, none displayed evident trends over the time or significant differences that may be explained by the different treatments (Figure 5.8b).

The predicted percentage of exotic spiders increased significantly ($p<0.01$) in summer-burn plots two and three years after the fire. In the last year of the study (four years after the fire) the difference was only between the control and the spring-burn plots (Figure 5.9). The mean observed percentage of exotic spiders increased from less than 10% in the years before the fire to over 50% in summer-burn plots in 2003 and 60% in spring-burn plots in 2004.

Only a few families, such as Linyphiidae and Gnaphosidae (Figure 5.10a and b) could be modeled adequately because of the extremely skewed distribution of the data on most families. Linyphiidae were significantly ($p<0.01$) less abundant in control plots than in fire treated plots before fire. After an increase over the years after the fire, linyphiids became more abundant in spring ($p<0.01$) and summer ($p<0.05$) burn plots in the last year of the study. Gnaphosidae showed significant but inverse differences between non-burnt and

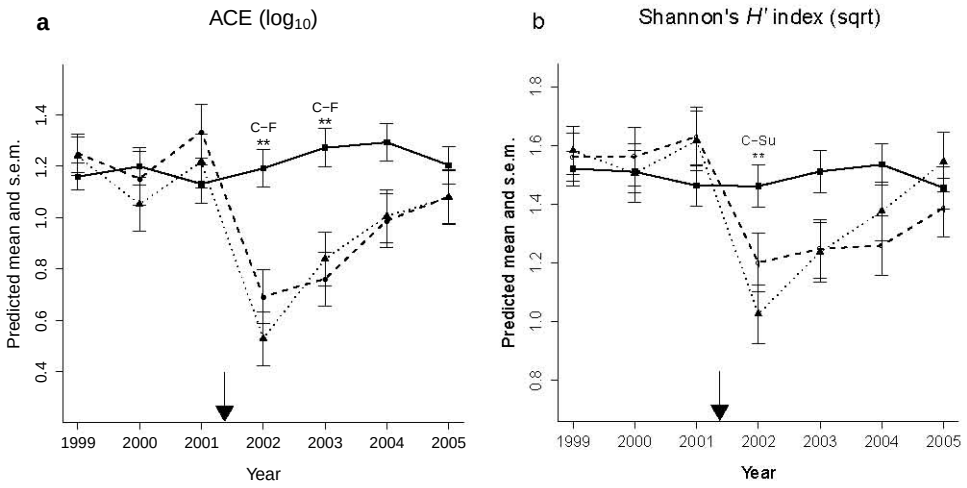

Figure 5.7: Values of (a) species ACE ($\log_{10}$) and (b) Shannon's H' diversity index (square root transformed) per m^2. Predicted means and their standard errors are shown. Solid lines represent numbers in control plots, dashed ones in spring-burn plots and dotted ones in summer-burn plots over the sampling years. Significant differences found through pairwise t-tests are represented by * for $p<0.05$ and by ** for $p<0.01$. Comparisons marked with "C", "F", "Sp" and "Su" indicate differences found between control plots, fire treated plots, spring-burn plots and summer-burn plots, respectively. The arrow indicates when spring and summer fires occurred (both between January 2001 and January 2002).

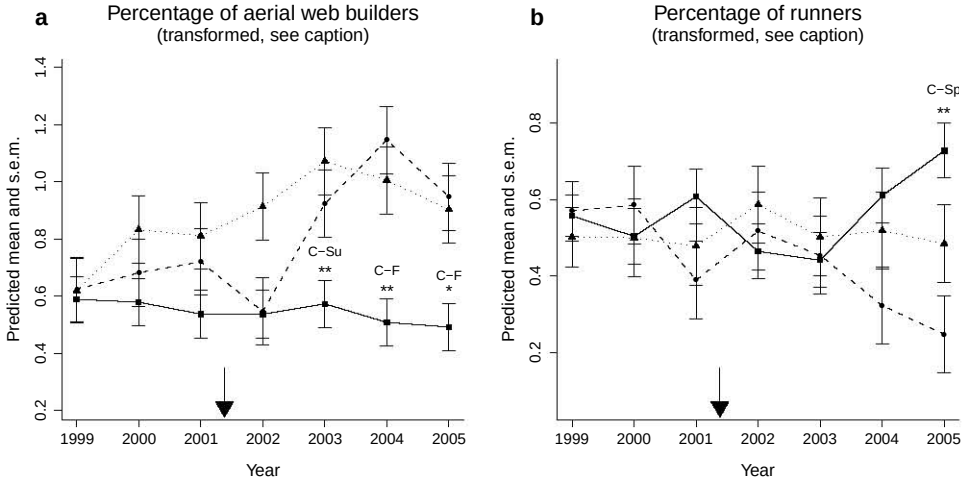

Figure 5.8: Percentage of (a) aerial web builders and (b) ground runners per m^2. Predicted means and their standard errors of transformed data ($\sin^2\theta$=(x + 3/8)/(n+3/4)) are shown. Solid lines represent numbers in control plots, dashed ones in spring-burn plots and dotted ones in summer-burn plots over the sampling years. Significant differences found through pairwise t-tests are represented by * for $p<0.05$ and by ** for $p<0.01$. Comparisons marked with "C", "F", "Sp" and "Su" indicate differences found between control plots, fire treated plots, spring-burn plots and summer-burn plots, respectively. The arrow indicates when spring and summer fires occurred (both between January 2001 and January 2002).

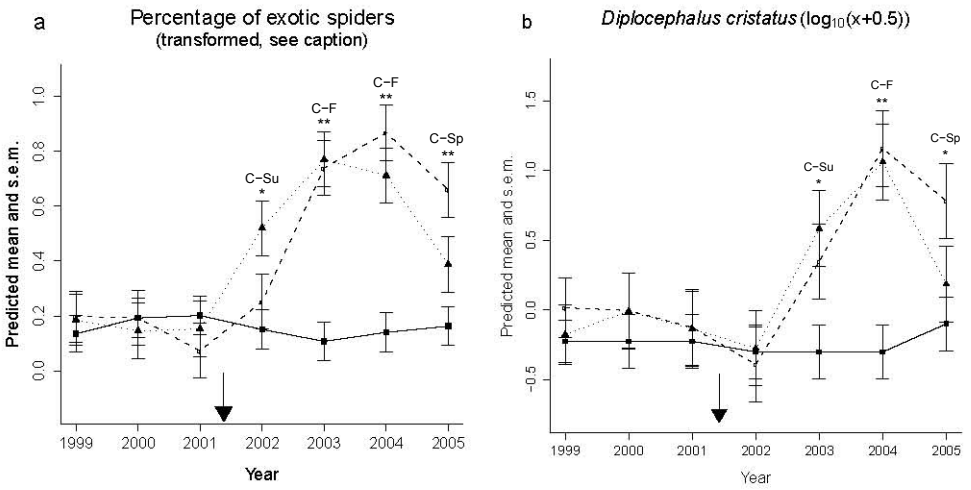

Figure 5.9: Percentage of (a) exotic spiders and (b) number of individuals of *Diplocephalus cristatus* ($\log_{10}$ +0.5) per m^2. Predicted means and their standard errors of transformed data ($\sin^2\theta$=(x + 3/8)/(n+3/4), for percentage of exotic species) are shown. Solid lines represent numbers in control plots, dashed ones in spring-burn plots and dotted ones in summer-burn plots over the sampling years. Significant differences found through pairwise *t*-tests are represented by * for $p<0.05$ and by ** for $p<0.01$. Comparisons marked with "C", "F", "Sp" and "Su" indicate differences found between control plots, fire treated plots, spring-burn plots and summer-burn plots, respectively. The arrow indicates when spring and summer fires occurred (both between January 2001 and January 2002).

burnt plots ($p<0.01$) before fire and between spring-burn and control plots ($p<0.05$) in 2003. Figure 5.11a, b and c show the observed means and their standard errors of families Lycosidae, Orsolobidae and Micropholcommatidae.

Among the species modeled, *Diplocephalus cristatus*, showed a clear trend over the time, with the species being significantly more abundant in summer-burn plots in 2003 ($p<0.05$) and in summer and spring-burn plots in 2004 ($p<0.01$) than in control plots (Figure 5.9). A significant difference ($p<0.05$) remained in the year 2005 between spring-burn and control plots.

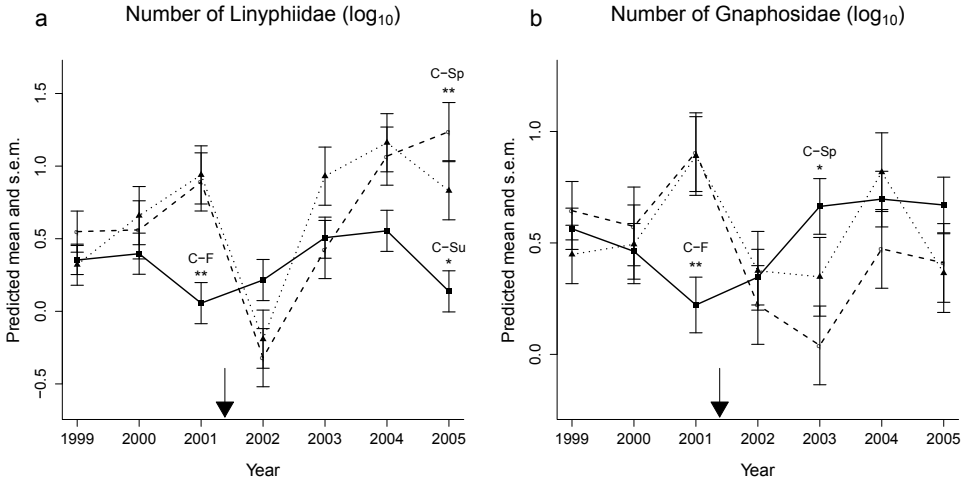

Figure 5.10: Number of (a) Linyphiidae ($\log_{10}$) and (b) Gnaphosidae ($\log_{10}$) per m^2. Predicted means and their standard errors are shown. Solid lines represent numbers in control plots, dashed ones in spring-burn plots and dotted ones in summer-burn plots over the sampling years. Significant differences found through pairwise t-tests are represented by * for $p<0.05$ and by ** for $p<0.01$. Comparisons marked with "C", "F", "Sp" and "Su" indicate differences found between control plots, fire treated plots, spring-burn plots and summer-burn plots, respectively. The arrow indicates when spring and summer fires occurred (both between January 2001 and January 2002).

5.4.3 Clustering Analyses

Ward's Minimum variance method

Ward's cluster analyses of the four different dissimilarity measures were relatively consistent in their results. The four matrices created clusters that separated plots with no previous event of fire — either control plots or spring and summer-burn plots before the fire — from plots after a fire.

The Euclidean dissimilarity matrix distinguished four main clusters, one of post-burn plots in the latest years of the study, two with plots with no fire and another one with plots in years immediately after a fire (Figure 5.12a). The clustering based on the Jaccard dissimilarity matrix separated plots according to the absence or presence of fire, and subsequently into groups representing plots from years shortly (2002 and 2003), immediately (March 2001) and in the latest years (2004 and 2005) after the fire (Figure 5.12b).

Similarly, Steinhaus index (S_{17}) clustered plots first according to presence or absence of fire, next into a group with plots with samples collected in the last years of the study, and then into a cluster of plots from the years right after

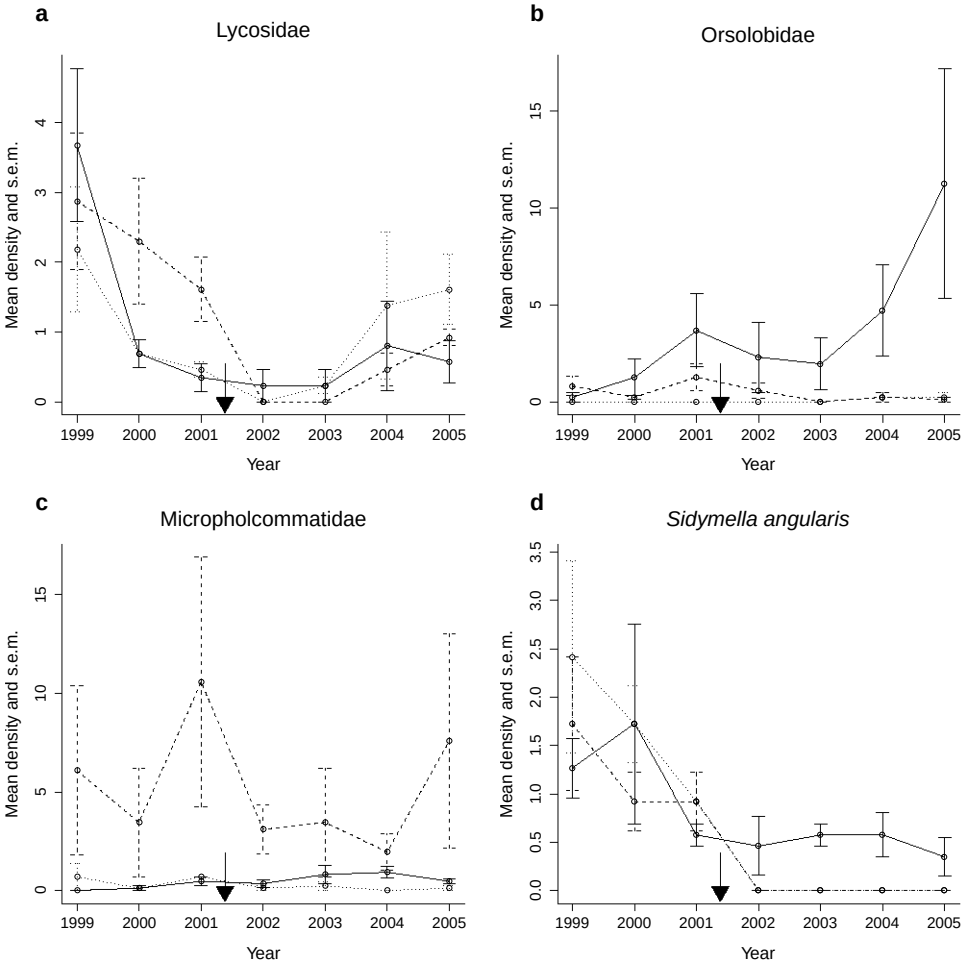

Figure 5.11: Observed number of individuals of families (a) Lycosidae, (b) Orsolobidae and (c) Micropholcommatidae and (d) the species *Sidymella angularis* per m^2. Means and their standard errors are shown. Solid lines represent numbers in control plots, dashed ones in spring-burn plots and dotted ones in summer-burn plots over the sampling years. The arrow indicates when spring and summer fires occurred (both between January 2001 and January 2002).

the fire and another from the following years (Figure 5.13a). Clusters created using the Sørensen index (S_8) also separated plots that had experienced fire recently and those in the later years of the study from others with no history of fire. However, the spider assemblage from summer-burn plots March 2001 — immediately after a summer fire — was closer to plots with absence of fire than those that had experienced it (Figure 5.13b).

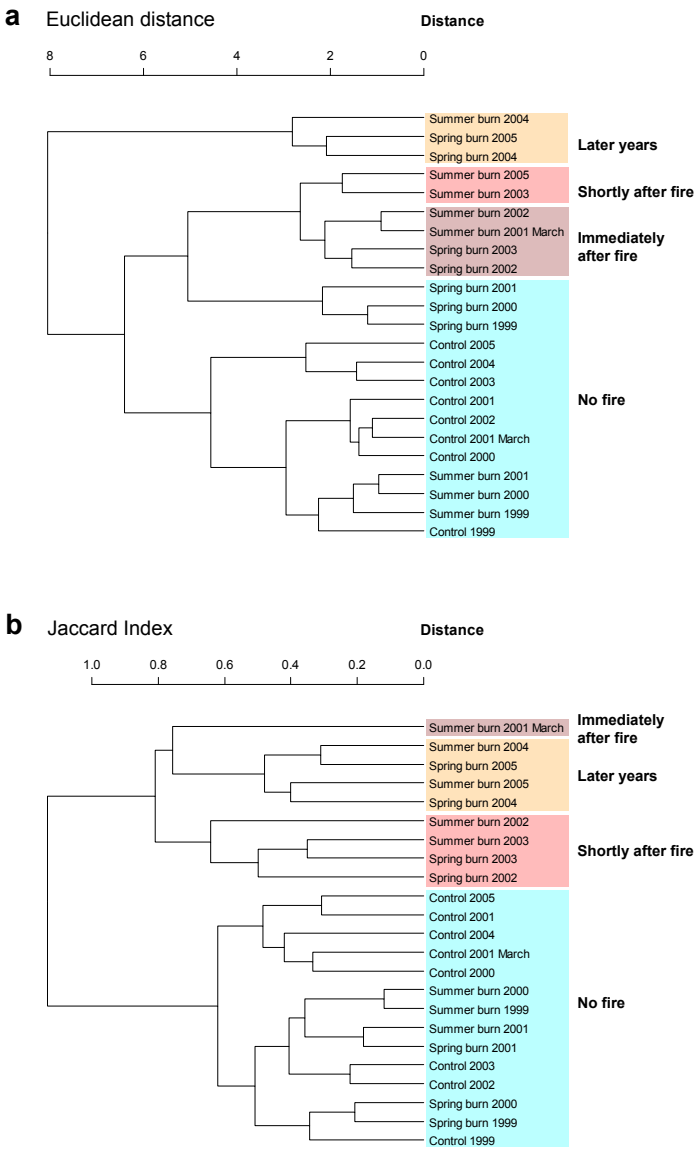

Figure 5.12: Ward's minimum variance clustering of assemblages from different years and treatments. a. Clustering based on Euclidean distances. b. Clustering based on Jaccard index.

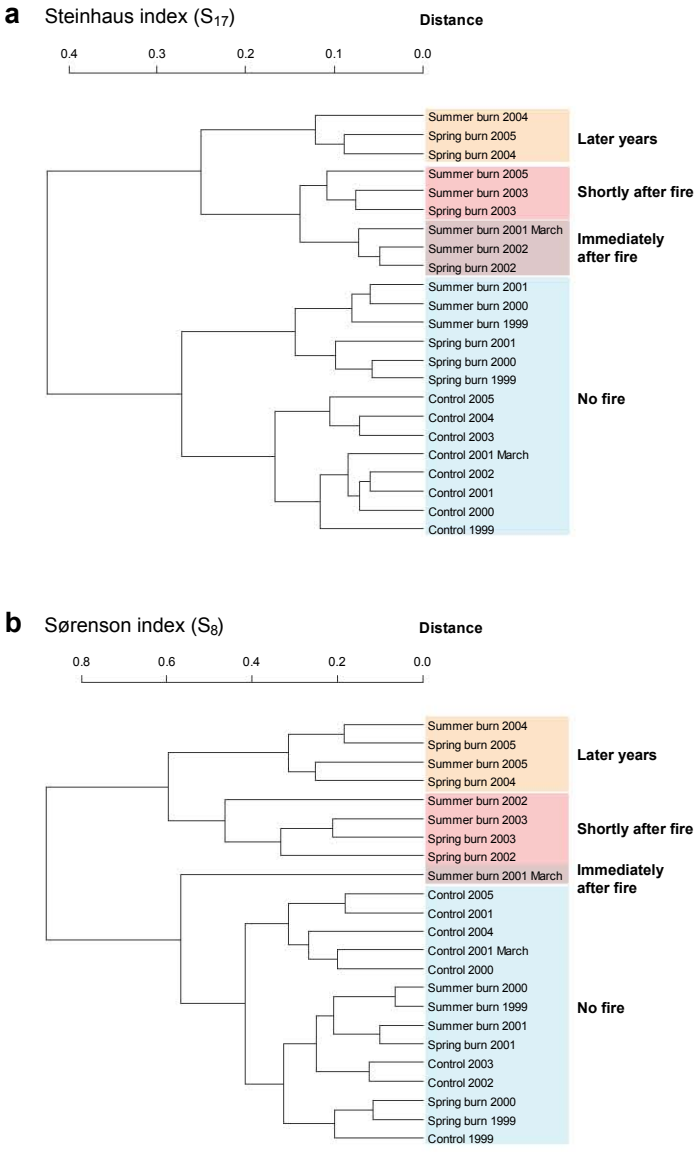

Figure 5.13: Ward's minimum variance clustering of assemblages from different years and treatments. a. Clustering based on Steinhaus coefficient (S_{17}). b. Clustering based on Sørensen coefficient (S_8).

K-means

Results showed that when spider assemblages of different treatments and years were forced into four, five or six groups through *K*-means analyses, the greater the number of groups, the more compact the set of groups was, as indicated by the Calinski and Harabasz value (C-H). An increase in the number of groups resulted in further separation of years into different groups, especially after the fire (Table 5.3 and Table 5.2). C-H values increased as the number of groups increased for both S_8 and S_{17} but they differed in the number of changes from one group to another over time. The partitioning based on S_8 grouped the last years of summer plots with pre-fire control and spring plots (Table 5.3). Clusters based on S_{17} were similar to those obtained though Ward's method, separating fire-free samplings from samplings after the fire and in the later years (Table 5.2).

Table 5.2: *K*-means partitioning of spider assemblages based on S_{17} dissimilarity matrix. Clustering of assemblages of each treatment and sampling date into four, five and six groups and their respective C-H pseudo-*F*-Statistic values are shown. Each colour represents a different group.

	Treatment	1999	2000	2001	2001 March	2002	2003	2004	2005	**C-H value**
4 gr.	Control	1	4	4	4	4	1	1	1	26.75464
	Spring	1	1	1	-	2	2	3	3	
	Summer	4	4	4	2	2	2	3	2	
5 gr.	Control	5	4	4	4	4	5	5	5	32.65483
	Spring	3	3	3	-	2	2	1	1	
	Summer	4	4	4	2	2	2	1	2	
6 gr.	Control	5	3	3	3	3	5	5	5	40.06211
	Spring	1	1	1	-	6	2	4	4	
	Summer	3	3	3	6	6	2	4	2	

5.4.4 Non-metric Multidimensional Scaling (NMDS)

The first two axes of the NMDS applied to the matrices based on the four dissimilarity measures suggested a separation of pre and post-fire samples based on community composition and abundance (Figure 5.14a, b, c and d). All four ordinations showed a gradient of plots on the first axis from control plots through pre-fire and recent post-fire plot to post-fire plots from later

Table 5.3: K-means partitions of spider assemblages based on S_8 dissimilarity matrix. Clustering of assemblages of each treatment and sampling date into four, five and six groups and their respective C-H pseudo-F-Statistic values are shown. Each colour represents a different group.

	Treatment	1999	2000	2001	2001 March	2002	2003	2004	2005	**C-H value**
4 gr.	Control	2	2	1	1	1	1	1	1	25.34693
	Spring	2	2	2	-	3	3	3	3	
	Summer	1	1	1	1	4	3	2	2	
5 gr.	Control	1	1	3	3	3	3	3	3	31.98037
	Spring	1	1	1	-	5	5	5	5	
	Summer	3	3	3	2	4	5	1	1	
6 gr.	Control	1	1	6	6	6	6	6	6	41.10377
	Spring	1	1	1	-	2	2	5	5	
	Summer	6	6	6	4	3	2	5	1	

years. In addition, the NMDS on the Jaccard dissimilarity index separated plot March 2001 from the rest of the pots, which was also indicated by the Ward's minimum variance clustering method based on the same matrix (Figure 5.14b).

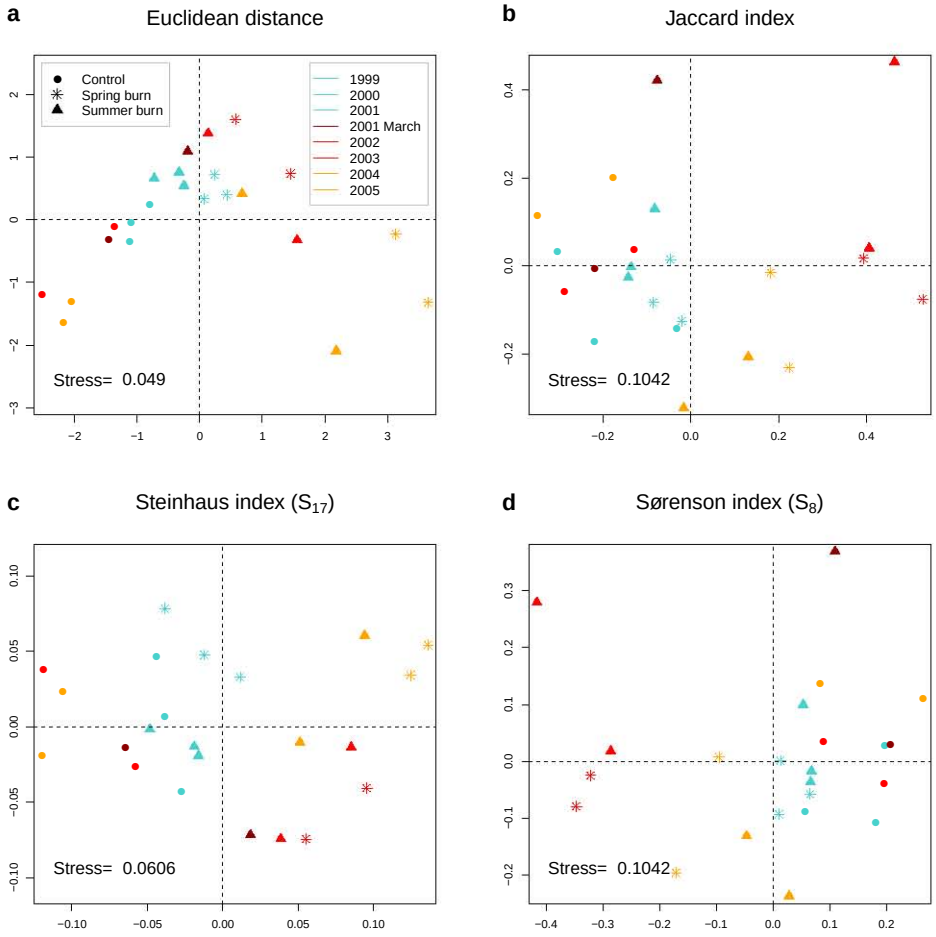

Figure 5.14: Non-metric multidimensional scaling of species assemblages for four dissimilarity measures, a. Euclidean distance. b. Jaccard index. c. S_{17} coefficient. d. S_8 coefficient. Circles represent control plots, stars spring-burn plots and triangles summer-burn plots. Blue, dark red, red and orange symbols represent pre-fire years, March 2001, 2002 and 2003, and 2004 and 2005, respectively.

5.4.5 Indicator species analysis (IndVal)

Clusters obtained from Ward's method on the S_{17} and S_8 coefficients provided the bases for identification of species characteristic of the different spider assemblages. Plots were grouped into four categories; no fire, immediately after fire, shortly after fire and later stage, although the plots included in each of the categories varied according to the coefficient used. Figure 5.15 shows the typology based on S_{17} and their respective characteristic species, with their indicator values.

Figure 5.15: IndVal analysis of characteristic species. Analysis was based on the typology of species assemblages obtained from Ward's clustering method on S_{17}. Only species significantly ($p<0.05$) associated with each cluster are shown. Indicator values of each species are shown within parentheses, with "max" indicating the cluster where a given species had its maximum value.

Although no species was found to be characteristic of the study area — represented by samples collected in all plots and years — some appear to have an affinity for particular years or stages. Plots with no history of fire were characterised by eight species, all of them native or most probably native: *Sidymela angularis*, *Hypoblemum* sp., *Parafroneta minuta*, Theridiidae sp.4, Mysmenidae sp.1, *Rinawa cantuaria*, Anapidae sp. and Salticidae sp.2, and all

of them with indicator values higher than 60. *Diplocephalus cristatus*, *Erigone wiltoni*, *Steatoda lepida* and *Araeoncus humilis* were significantly associated with plots where fire occurred, with the former three having the highest indicator values for this assemblage. The two species with the highest indicator values — *D. cristatus* and *E. wiltoni* — were exotic species.

Within the cluster of plots affected by fire, only species characteristic of the later stages in the timeline were identified. This group of plots from the later years of the study were characterised by *D. cristatus*, *A. humilis*, *Anoteropsis hilaris*, *S. lepida*, *E. wiltoni*, *Maorineta tumida*, *Nauhea tapa* and *Anzacia gemmea*, three of which — the first, second and fifth — are exotic species and the remaining widespread species.

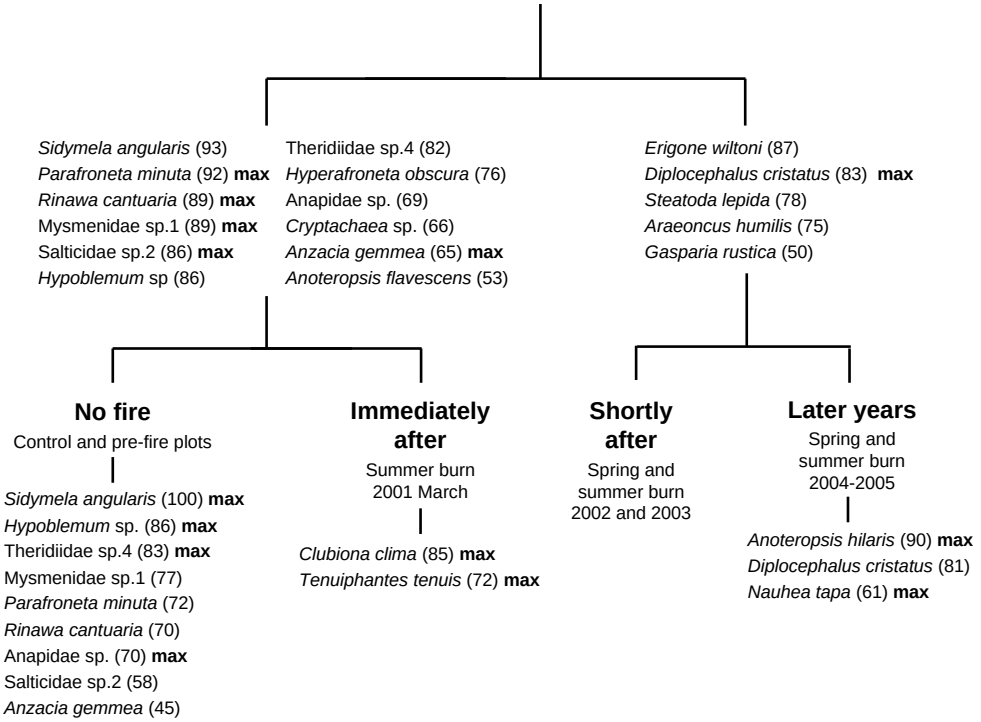

Figure 5.16: IndVal analysis of characteristic species. Analysis was based on the typology of species assemblages obtained from Ward's clustering method on S_8. Only species significantly ($p<0.05$) associated with each cluster are shown. Indicator values of each species are shown within parentheses, with "max" indicating the cluster where a given species had its maximum value.

The typology based on S_8 identified similar indicator species for each category (Figure 5.16). This typology grouped plots in a chronological order that permitted the separation of the summer-burn plots from March 2001, which, in

turn, allowed the detection through IndVal of the species *Clubiona clima* and *Tenuiphantes tenuis* as characteristic of the spider fauna present immediately after a summer fire.

5.5 Discussion

5.5.1 Number of individuals

Modelling of response variables linked with spider diversity found trends that agree with previous findings. Although most variables fluctuated over the years, there were significant decreases in the years following the fire in spring and summer-burn plots. Number of individuals decreased in the year immediately after the fire but recovered to pre-fire numbers within three years. This severe initial drop in the overall number of spiders after fire is commonly observed (Andersen and Muller, 2000; Huhta, 1971; Langlands et al., 2006) and associated with direct death of spiders by burning during the fire and indirect effects caused by changes in microclimatic conditions, spatial structure, dispersal, life cycle, competition, nutrition and increase in predation (Hartley et al., 2007; Huhta, 1971; Swengel, 2001).

Fire can change succession by affecting plant productivity (Collins, 2000; Force, 1981) and regeneration of shrubby vegetation (Covington et al., 1997; Gibson and Hulbert, 1987), which, in turn, can affect spider abundance (chapter 4). The predator-prey relationship between spiders and invertebrate herbivores establishes their connections within trophic webs (Hunter and Price, 1992; Moran and Scheidler, 2002) and determine the changes in their populations through top-down and bottom-up interactions. Therefore, fire can decrease spider abundance through direct mortality, the reduction in the physical complexity of their habitat and the reduction in the abundance of prey.

Although there was a marked decrease in the number of spiders after the fire, they were present almost immediately after the fire and they recovered rapidly in the following years. The presence of spiders immediately after burning (Ferguson et al., 2003) and their quick recovery afterwards (Abbott et al., 2003) have both been reported. Spiders' resilience to fire may be explained partly by the annual fluctuation in their populations as well as their ability to avoid fire by escaping it (Hartley et al., 2007) or by finding refuge in soil or

non-flammable plant debris (Warren et al., 1987). The recovery of spider populations only two years after the fire is probably due to dispersal by ballooning (Wise, 1993), especially by members of the family Linyphiidae. Colonisation of burnt sites depends on their patchiness and distance from unburnt areas (Barratt et al., 2009). The burnt plots of this study were surrounded by unburnt areas that could act as a source of colonisation for new populations of spiders. Given the relatively small size of plots, the rapid recovery of spider populations in the burnt plots could be explained by the proximity from unburnt areas to any point within the plots.

Although an increase in the number of spiders over time after a fire has often been observed (Haskins and Shaddy, 1986; Merrett, 1976), this has not always been so (Niwa and Peck, 2002) as it depends on the time scale and the annual fluctuations that may occur in the years following the fire (Langlands et al., 2006). Such fluctuations may also explain the fact that the total numbers of spiders, species, families, linyphiids and gnaphosids in control plots were significantly lower than in fire plots in one of the years before the fire. Having observed pre-fire differences between control and fire plots, it may be argued that stochastic variation may be a confounding factor when measuring differences between treatments. However, the general decrease in all response variables after the fire and the increasing trends over time appear to indicate that fire is a major driver of the changes in spider diversity.

5.5.2 Number of species and families

Differences in diversity between treatments detected by k-dominance curves were supported by generalised linear mixed models. The significant differences in the number of species and families between control and treatments lasted for several years after the fire but they appeared to increase over time. Although this upward trend has been shown before (Huhta, 1971; Urones and Majadas, 2002), the numbers obtained in this study did not reach the plateau observed in studies covering between 9 and 24 years after a fire (Brennan et al., 2006; Moretti et al., 2002). Therefore, reaching a plateau in the number of species and families may require a longer sampling period. On the other hand, values of ACE and Shannon's H' diversity suggested that the overall diversity

may have reached levels similar to those in pre-fire years during the course of this study. As with the number of spider individuals, a greater habitat complexity achieved through changes in the vegetation and litter may account for an increase in species and family richness over time (Brennan et al., 2006). Over the years, the physical structure of the vegetation may increase in complexity, which could, in turn, affect spider composition (Greenstone, 1984; de Souza Teixeira and Parentoni Martins, 2005) by providing greater three-dimensional space for webs and creating favourable microclimatic conditions (see chapter 4).

5.5.3 Guild and family responses

As mortality depends on the specific mobility of the members of each family and their probability of exposure to flames (Swengel, 2001), certain families will be more susceptible to fire. Likewise, the effects of fire will differ depending on the specific environmental requirements of each species or family. Ground active spiders may be affected by changes in plant cover (Riechert and Reeder, 1972), whereas web builders will depend more on the three-dimensional structure of the vegetation (Urones and Majadas, 2002). Also, web building families such as Linyphiidae may colonise and increase in number in burnt areas after a fire (York, 1996) due to their adaptations for long-distance dispersal.

Lycosidae, Araneidae, Theridiidae and Linyphiidae are generally considered pioneers after a fire because of their dispersal ability (Brennan et al., 2006; Merrett, 1976; Riechert and Reeder, 1972). The results obtained in this study show a dominance of Linyphiidae, thanks partly to an increase in the populations of exotic species with great dispersal ability and perhaps also because of the lack of adaptation of most native spiders to fire. The species associated most strongly with fire treated plots were either aerial web builders or ground active spiders, both known as groups of successful colonisers because of their ability to disperse on the ground or through ballooning. Interestingly, Orsolobidae, a family linked to moist conditions and vegetation (chapter 4), was, though in low numbers, present in the burnt plots immediately after the fire. Although moist specialists decrease in or move away from burnt areas (Riechert and Reeder, 1972; York, 1996), Orsolobids have been seen in Aus-

tralian jarrah forests after fire (Brennan et al., 2006). The presence of these spiders may be explained by undiscovered adaptations to avoid disturbance, such as underground retreats, and it may deserve more investigation.

Litter dwelling invertebrates, such as spiders of the family Hahniidae, have potential as bioindicators for changes in vegetation (chapter 4). Since fire destructs the habitat of hahniids through litter removal, they can also be used as indicators of fire intensity (Henig-Sever et al., 2001). However, no clear trends were detected from this study in the number of hahniids in the years after the fire.

5.5.4 Changes in spider assemblages

There were clear differences in spider diversity between control and fire treated plots after the fire. Although annual variation can have an effect on spider composition and abundance (Langlands et al., 2006), the distinction between fire exposed and non-exposed plots and the subsequent chronological grouping of years shown by the different clustering analyses made the impact of fire clear. Fire can affect composition and relative abundance of spiders by changing environmental conditions and habitats shared by different spiders and thus altering the competition or interactions between them (Ehmann and MacMahon, 1996; Moretti et al., 2002). These changes may happen through modification in the structure of the vegetation (Riechert and Reeder, 1972) as well as amount of litter (York, 1996).

Modification of spider assemblages was indicated by the initial separation of plots according to the presence or absence of fire by Ward's method on S_{17} coefficient and Jaccard index, and the chronological separation of clusters by k-means. Differences in assemblages were confirmed by NMDS, which also suggested an increase in such differences as time went on. Similar changes in assemblages in the years following a fire have been reported in other systems (Brennan et al., 2006). Changes in vegetation and microclimatic conditions in the years following the fire may explain changes in spider assemblages as these vary according to the specific habitat requirements of the different families or species (Huhta, 1971; Merrett, 1976). Although these changes may be part of a succession towards the recovery of the populations of species belonging to the

area (Huhta, 1971), the final stage of a possible succession was only indicated by one cluster method. K-means clustering on S_8 showed signs of recovery in the last years of the study, when the assemblages in summer-burn plots became similar to pre-fire plots. Since S_8 is a presence/absence coefficient, this may suggest that although the populations of the species present before the fire had not recovered to previous numbers, they had at least returned.

5.5.5 Characteristic species

Variation in spider assemblages was indicated by the characteristic species of the different clusters detected by IndVal. Two species were identified as characteristic of spider assemblages present immediately after the summer fire, *Tenuiphantes tenuis* (Linyphiidae) and *Clubiona clima* (Clubionidae). This association may be explained by their seasonal activity, with a peak in autumn, their preference for the large open areas left by the fire, or perhaps both. Although there is not enough information available in the literature to explain the response of *C. clima*, it is obvious that both species must be very efficient in their dispersal (Merrett, 1976) and therefore adapted to colonising disturbed areas rapidly. This is certainly the case for *T. tenuis* as it is one of the most abundant colonisers of experimental grass swards and recently disturbed areas thanks to its effective dispersal through active ballooning (Haughton et al., 2001; Merrett, 1976). Moreover, the life history of *T. tenuis* allows it to survive in areas under regular disturbance and stay active all year (Topping and Sunderland, 1994). As a result, *T. tenuis* has a large geographical range covering a variety of different habitats (Topping and Sunderland, 1998) and is among the most common linyphiids in British, central European and New Zealand agroecosystems (Haughton et al., 2001; McLachlan and Wratten, 2003; Schmidt and Tscharntke, 2005; Vink et al., 2004).

Native species had a greater association with areas with no history of fire whereas exotic species favoured burnt sites, particularly in the last two years of the study. This suggests that most native species may not be well adapted to frequent fire in tussock grasslands or at least not as well as exotic species. However, confirming these conclusions requires longer term experiments and the study of the effects of fire at different frequencies. Furthermore, when

investigating or speculating about adaptations of tussock species, it is necessary to consider what vegetation types have been dominant in the past in the study areas, how frequent fire has been there, and, whether the resulting current tussock spider assemblages are representative of past assemblages.

Vegetation can determine fire frequency and vice versa, and their conditions and frequency have varied in New Zealand over the last centuries. Fire regime has gone from relatively low frequency in pre-human times to an increase in times of Polynesian and later European settlements Ogden1998, changing the dominant vegetation types as a result. Estimations by McGlone (McGlone, 2001) for the time before human arrival (the mid to late Holocene, between 5000 to 800 BP) included the Lammerlaw range within the zone dominated by close forest. However, debate continues over the extent of grasslans/ shrubland and the fire frequency in that and other areas in the south of the South Island Bond2004, Rogers2007.

Spider assemblages have probably adjusted to new vegetation types and fire regimes as they arose. As conditions changed, populations would go through bottlenecks, some species would become rare and certain species would go locally or totally extinct and be replaced by others. Such processes would occur through selection mechanisms dependent on species characteristics, such as dispersal ability or habitat requirements. As Polynesians used fire to clear woodlands, the area covered by native grasslands expanded, which may have caused an increase in the abundance and distribution of species adapted to tussock habitats. Finally, in the last two centuries, the arrival of exotic species with a better ability to re-colonise and exploit resources in post-fire conditions may have driven few native species to become rare, including some of the rare species found in this study.

Therefore, the response of spider assemblages shown in this study should be considered and understood in the current context of the study site, namely a tussock dominated area with periodic human induced fires.

5.5.6 Native vs. exotic species

The increment in the number of web building spiders after the fire can be explained by the increase in a single species, the linyphiid *Diplocephalus*

cristatus. This European species is widely established in New Zealand and is present in disturbed areas (Topping and Lövei, 1997), at high altitudes in tussocks above the bushline (Forster et al., 1988), and has been recorded in modified native shrublands in the Rock and Pillar range (Derraik et al., 2005), close to the study area.

The increase in *D. cristatus* contrasts with the disappearance of *Sidymella angularis* (Thomisidae) from the burnt areas. Although the low numbers of *S. angularis* before the fire and in control plots does not allow to state that this drop may be due to annual fluctuations in its population, the total absence of adult individuals after fire suggests a severe effect. Close association with specific plants can make burnt areas unsuitable for certain invertebrates (Wright and Samways, 1998, 1999), and *S. angularis* may be an example, as it probably depends on certain plant species for camouflage (Brennan et al., 2006). These results are in concordance with findings that Thomisidae are more abundant in unburnt sites where conditions are more stable and plant species that they are associated with are present (Niwa and Peck, 2002).

5.5.7 Spring vs. summer fire

The time or season that a fire occurs in can affect abundance of arthropods but the effects differ depending on each taxon. Populations of spiders (Andersen and Muller, 2000) and Coleoptera (Blanche et al., 2001) decline markedly after a fire late in the dry season. More specifically, in New Zealand tussock grasslands, summer fire did not appear to have a greater effect on beetles, or the overall number of invertebrates than spring fire (Barratt et al., 2009).

In this study there were no significant differences in most response variables related to spider diversity between spring and summer-burn treatments. Only the number of species in the year following the fire was different for the two fire treatments, with numbers being lower in the summer-burn treatment. Given that the temperature at soil surface during fire was higher in spring plots (500–1010°C) than in summer burn plots (300–500°C), and the spring fire occurred three months and summer fire ten months before the sampling date, such difference in species number may be explained by the characteristics of the spider communities and their habitats at the time of the fire, rather

than the characteristics of the fire itself. The short term difference between the two fire treatments may be due to the activity level of spiders at the time of the fire and their habitat requirements, such as amount of litter (Andersen and Muller, 2000) for web construction or presence of specific plant species for camouflage (Riechert and Reeder, 1972). Seasonality in activity and reproduction of spiders is certainly a factor to consider when measuring the effects of disturbance in tussock grasslands. The number of adults — reproductively active individuals — increases in tussock grasslands as summer progresses (unpublished data). Therefore, a disturbance at the time of the year when adults are more abundant - end of summer in this case - may have a greater effect on future populations.

Differences between spring and summer fire treatments over time were shown by k-dominance curves and supported by Ward's clustering on S_8 coefficient. Years in spring-burn treatment were separated into distinct groups whereas in summer-burn plots they changed more gradually, reaching a recovery stage in the last years of the study. Despite a greater initial effect by fire, it appears that spider communities of areas affected by summer fire go through a more rapid recovery, which is, perhaps, related to the lower soil surface temperature in summer fire. As spider communities in tussock grasslands respond to structural and compositional characteristics of the vegetation (chapter 4), different changes in assemblages over time may reflect differing changes in vegetation. Changes in the plant community, in turn, may be determined by the intensity of fire and its effects in different seasons on the growth or reproductive stages of plants. Alternatively, changes in spider assemblages over time may be due to random variation across the plots.

5.5.8 Conclusions

The study presented here had certain limitations, especially concerning the number of replicates and sample extraction. A greater number of replicates and sites may have allowed removing the effect of temporal variation in spider assemblages. In addition, there are questions about the consequences of storing samples at low temperatures before invertebrate extraction. Nevertheless, this study shows a number of clear responses of spiders to fire and trends

in their diversity over time. Addressing more specific questions of whether the differences between the different fire treatments are due to differences in vegetation, prey availability or stochasticity requires the long term collection and analysis of additional botanical and environmental data.

5.5.9 Recommendations for management

Burning of grasslands can be compatible with insect diversity conservation in certain circumstances and provided that it is carried out in the cool season (Panzer and Schwartz, 2000). However, this study demonstrates the severe effect of fire on spider communities of semi-modified native tussock grasslands of Central Otago. Based on the results of this study, we recommend:

- Reduce the frequency of fire as much as possible to allow the recovery of populations of native invertebrate species and prevent the colonisation and establishment of exotic species.

- Combination of data from studies at different sites and regions, which would allow extrapolation of conclusions and generalisation of recommendations. Studies at multiple scales could help investigate colonisation processes and edge effects in burnt areas.

- The creation and promotion of long term ecological studies to further investigate the effects of fire. Studies looking at the effects of fire in different seasons and at different frequencies could answer questions about their effects on the flora, fauna and the environmental characteristics of tussock grasslands.

Summarising, this study made the following findings; one, fire has an overall detrimental effect on spider diversity in tussock grasslands; two, fire determines the trends or changes in tussock grassland spider assemblages over time; three, the number of spiders belonging to exotic species, which are often colonisers of disturbed areas, increases in the years after a fire in tussock grasslands; and four, compositional changes in spider assemblages over time after a fire are dependent on the season in which the fire occurs.

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Chapter 6

Thesis summation

This thesis establishes the basis for the study of the diversity and ecology of spiders in New Zealand tussock grasslands by providing crucial information for future studies, from methodological aspects to data on the interaction of spiders with their environment.

6.1 Sampling Methodology

Maximisation of resources through the selection of efficient sampling methods is fundamental for the success of any ecological study (Cardoso et al., 2008; Coddington et al., 1991). **Chapter two** evaluated the effectiveness and efficiency of a set of sampling methods in tussock grasslands. Pitfall trapping, a method that aims to collect ground active spiders, was found to be the most appropriate sampling method as it collected a large number of spider individuals and the greatest number of species.

The efficiency of a sampling method depends on many factors, including the physical structure of the vegetation and the area or layer of vegetation that the sampling method covers. The dense physical structure of certain species of tussock, such as *Chionochloa rigida*, can hinder the use of methods such as suction sampling, as spiders can find refuge at the dense bases of the tussocks.

Although pitfall trapping is recommended for the collection of spiders in tussock grasslands, other methods, such as beating, should be considered as they target species present at different layers of the vegetation and may capture a greater proportion of adults. The efficiency of sampling methods is partly determined by the proportion of adults as they are the only life stage

that can be reliably identified morphologically. Nevertheless, future developments in molecular specimen identification may help overcome morphological impediments (**chapter three**).

6.2 DNA for completion of ecological data

Chapter three demonstrated the potential of DNA taxonomy and DNA barcoding as important tools for ecological studies. Matching of genders of undescribed species, discovery of possible new species and categorisation of certain specimens as exotic through the use of molecular tools can add valuable information to ecological data, as exemplified by the use of molecular information from **chapter three** to supplement the data used in the ecological studies of **chapter four** and **chapter five**. Relatively simple cluster analyses, such as neighbour joining trees, on sequences of the mitocondrial gene region cytochrome *c* oxidase subunit I (COI) provided evidence of the potential of this technology in ecology. Certain taxa, however, may pose unexpected difficulties when using DNA taxonomy or barcoding, as in the case of the genus *Orepukia*, which revealed similar genetic distances between specimens of the species *O. poppelwelli* and specimens belonging to two clades of the congeneric species *O. orophila*.

An assessment of general mixed Yule-coalescent (GMYC) models (Pons et al., 2006a) for specimen identification suggested that this method can fail when taxa included in the analyses are too distantly related, when speciation-coalescence branching rates vary largely across taxa or when the genetic distance between certain congeneric species is relatively short. Nevertheless, the results of this study show that molecular tools can be included in ecological studies to help answer questions about community ecology.

6.3 Diversity patterns

Chapter four represents one of the first complete assessments of the spider assemblages of a tussock grassland of New Zealand and the biotic and abiotic factors that drive them. Variation in spider assemblages in tussock grasslands is driven by changes in vegetation, which, in turn, is driven by a number of environmental factors, including soil moisture. The resulting patch-

iness of spider communities within the ecosystem is, therefore, a consequence of the response of specific spider taxa to habitat conditions determined by a vegetation mosaic. For instance, spider taxa, such as orsolobids, associated with moist conditions are more abundant in "islands" of wetland vegetation scattered in tussock grasslands.

The hypothesis that physical structure of the vegetation has a larger effect on spider diversity than the botanical composition (Dennis, 2003; Dennis et al., 2001; Uetz, 1991) was tested for the first time in native tussock grasslands. Both physical structure and botanical composition showed significant relationships with various response variables related to spider diversity, with increased spider diversity with greater tussock cover and plant diversity. When quantifying the effects on spiders, a similar number of species and families displayed a significant relationship with either factor (Figure 4.11).

Two species were identified as potential indicator species for changes in the ecosystem. *Anoteropsis hilaris* and *Anoteropsis flavescens* were more abundant in areas with less and more tussock cover, respectively. Being relatively easy to capture and identify, *A. hilaris* and *A. flavescens* could become the focus of standardised protocols for monitoring changes in tussock grasslands under restoration. As with sampling methodology, the selection of particular target taxa that provide valuable data can optimise resource use and increase the efficiency of ecological studies. The results of this study showed that pitfall traps and turf extraction provide similar results, which favour the selection of the former as it is far less time and resource consuming; this is congruent with the results of **chapter two**.

6.4 Effects of fire

Spider assemblages respond to natural changes in their physical environment as well as changes caused by anthropogenic disturbances (Buddle et al., 2000; Marc et al., 1999; Ziesche and Roth, 2008). **Chapter five** evaluates the effects of fire, a common disturbance in tussock grasslands.

Measures of spider diversity, such as total number of individuals, number of species and families and diversity indices, decreased remarkably after a fire. Although the total number of spiders recovers rapidly, the number of species

and families continued to be affected in the mid- to long-term and had not fully recovered four years after the fire. Changes in spider composition also showed a trend over time, with assemblages going through a series of successional stages.

The hypothesis that summer fires are more detrimental than spring fires (Barratt et al., 2009) was tested by including two experimental treatments. Summer fire plots had significantly fewer species and families than spring fire plots in the year immediately after the fire. Plots that experienced fire in summer had greater variation in assemblages over the years following a fire. Although plots affected by summer fire suffered a more marked effect in the short term, their recovery through different succession stages appeared to occur more rapidly.

Exotic and native spider species responded differently to fire. Species associated with previously burnt plots were aerial web building exotic species. The increase in the number of such species can be explained by their exceptional dispersal ability and capacity to colonise recently depopulated habitats. The study also shows that, at least in the short term, spiders native to New Zealand tussock grasslands are not adapted to disturbances, such as fire, unlike opportunistic exotic species. This suggests that natural fires were infrequent in New Zealand before the arrival of humans.

6.5 Management recommendations

This project produced valuable information for land or conservation management. The combination of the evaluation of data collection methods, the analyses of factors driving diversity and the assessment of the effects of human activities allows me to make the following recommendations:

- When designing protocols for surveys or ecological studies on tussock grassland spiders — and probably other ground active arthropods — pitfall traps should be included as one of the sampling methods.

- If resources allow it, DNA identification methods should be considered as they will provide information to complete ecological data and avoid mistakes in conclusions drawn from incorrect specimen identification.

- Monitoring programs, such as those used in restoration projects, should consider using spiders, and more specifically, indicator species such as *Anoteropsis hilaris* and *A. flavescens*, for the assessment of physical and botanical changes in tussock grasslands.

- Fire as a management practice should be limited as much as possible in order to avoid long term detrimental effects on invertebrate fauna and limit the opportunity for the dispersal of potentially invasive exotic species.

6.6 Directions for future research

Spiders have been the subject of much ecological research because of their function as generalist predators (Wise, 1993). However, New Zealand spiders, and more particularly communities from native ecosystems, are little known. Instead, spiders have been the focus of predominantly taxonomic research, probably due to the need to first describe the large number of unknown native species and also due to the low number of spider ecologists in the country.

Although I consider this thesis as a step towards understanding the biotic and abiotic interactions of spider communities in tussock ecosystems, there are still numerous questions to answer. Here I present some of these questions, which will hopefully stimulate further research on spider ecology.

1. Native grasslands at other latitudes and altitudes should be compared to the tussock grasslands studied here in order to fully understand the processes driving spider diversity in native ecosystems with open vegetation in New Zealand and to determine how universal my results are. Climatic conditions determine plant communities and therefore, different spider assemblages should be found to respond differently.

2. The exploratory study of environmental factors that affect spiders should be corroborated by experimental data looking at specific processes that underpin them, as well as other potential drivers, such as levels of humidity and temperature within tussocks.

3. Long term projects on the effects of fire should be conducted in managed grasslands. The information obtained from such studies would

provide objective data with which to make long term management decisions. Specific questions could include the difference between spring and summer fire over longer periods of time and the importance of fire frequency. This kind of research could help determine whether fire is a frequent natural disturbance in tussock grasslands.

4. Experimental studies could also answer questions related to the impact of exotic spiders in tussock grasslands. Some species may be replaced after a disturbance in the short term, and perhaps even in the long term. The mechanisms through which species turnover or replacement occur may be explained by experiments on interactions between species.

5. Finally, more taxonomic work — both morphological and molecular — is necessary to solve the "taxonomic impediments" that ecologists frequently encounter when identifying specimens. Approximately 40% of the species present in New Zealand have not yet been described, which constrains the ability to sort and identify large number of samples, which are common in ecological studies. The solution to this problem will require the collaboration between ecologists, who collect large numbers of specimens, and taxonomists with the necessary expertise to describe species.

There are undoubtedly still many surprising and exciting ecological processes that spiders take part in that await discovery. I hope that this thesis will encourage other researchers to continue with this work.

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Appendix A

Ellangowan Scenic Reserve: Spider species

Table A.1: List of spiders species collected through foliage beating, pitfall trapping, suction sampling and emergence trapping.

	Sampling method			
	Foliage beating	Pitfall traps	Suction sampling	Emergence traps
Amaurobiidae				
Paravoca otagoensis Forster & Wilton, 1973	-	X	-	-
Amphinectidae				
Maniho ngaitahu Forster & Wilton, 1973	-	X	-	-
Clubionidae				
Clubiona blesti Forster, 1979	X	-	-	-
Desidae				
Hapona otagoa (Forster, 1964)	-	X	X	-
Laestrygones sp.	-	-	X	-
Gnaphosidae				
Zelanda erebus (L. Koch, 1873)	-	X	-	-
Hexathelidae				
Porrhothele antipodiana (Walckenaer, 1837)	-	X	-	-
Linyphiidae				
Diplocephalus cristatus(Blackwall, 1833)	-	X	-	-
Erigone wiltoni Locket, 1973	X	-	-	-
Lycosidae				
Anoteropsis hilaris (L. Koch, 1877)	-	X	-	-

Continued on next page

Table A.1 – continued from previous page

	Foliage beating	Pitfall traps	Suction sampling	Emergence traps
Malkaridae				
Malkaridae sp.	-	X	-	-
Micropholcommatidae				
Micropholcommatidae sp.1	-	X	-	-
Micropholcommatidae sp.2	-	X	-	-
Micropholcommatidae sp.3	-	X	-	-
Mysmenidae				
Mysmenidae sp.1	-	-	X	-
Mysmenidae sp.2	-	-	X	-
Nemesiidae				
Stanwellia kaituna (Forster, 1968)	-	X	-	-
Pisauridae				
Dolomedes minor L. Koch, 1876	-	X	-	-
Salticidae				
Salticidae sp.1	-	X	X	-
Salticidae sp.2	-	X	-	-
Stiphidiidae				
Cambridgea quadromaculata Blest & Taylor, 1995	-	X	-	-
Synotaxidae				
Pahora sp.	-	X	-	-
Theridiidae				
Moneta conifera (Urquhart, 1887)	-	-	X	-
Theridiidae sp.1	-	X	X	X
Theridiidae sp.2	-	X	-	X
Thomisidae				
Thomisidae sp.	-	X	-	-
Total number of species	20	7	2	2

Appendix B

Additional data on molecular analyses

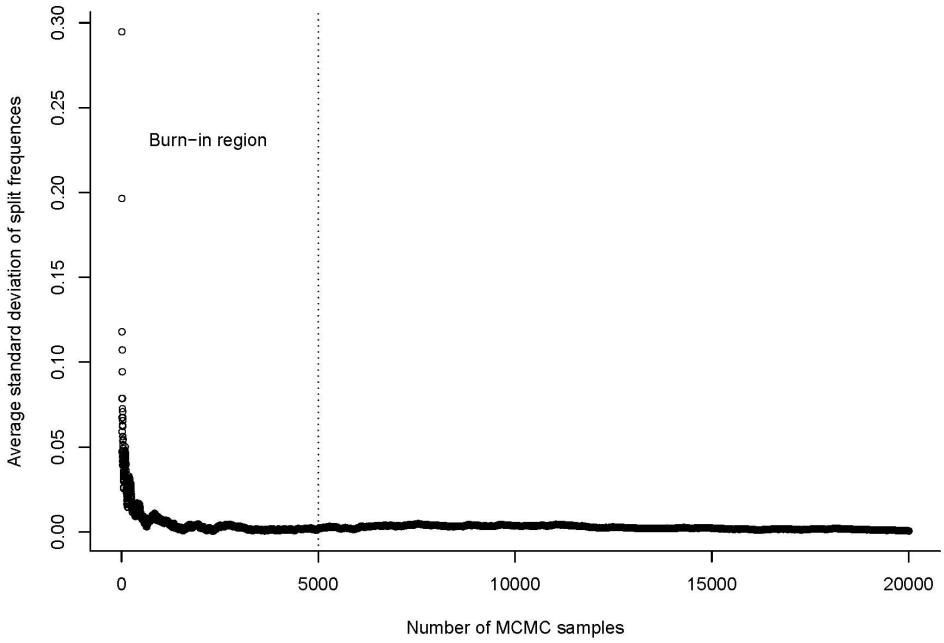

Figure B.1: Average standard deviation of split frequencies for sampled tree. Each tree was sampled every 1000 generations.

Table B.1: Sequence codes, species, sex, sampling locations and dates, and used sampling methods or names of collectors of the sequenced specimens. Abbreviations; j.: juvenile, TE: Turf extraction, PT: Pitfall trap, Chch: Christchurch, AK: Auckland, WN: Wellington, NZ:New Zealand. Collection site and plot numbers represent locations specified in chapter 4. Specimens collected with pitfall traps and turf extraction were captured by the author of the thesis following the methods described in chapter 4.

Sequence code and species	Sex	Collection site/plot	Collection date	Method or collector
9716. *Hypoblemum* sp.	♂	5/1	19-I-2008	TE
9717. *Hypoblemum* sp.	♀	5/2	19-I-2008	TE
9715. *Hypoblemum* sp.	♀	5/1	3-III-2008	TE
9714. *Hypoblemum* sp.	♀	5/1	17-III-2008	TE
1011. *Hypoblemum* sp.	♀	3/3	2-II-2008	TE
1012. *Hypoblemum* sp.	♂	1/1	14-XII-2008	TE
1013. *Hypoblemum* sp.	♀	4/1	15-XII-2008	TE
1014. *Hypoblemum* sp.	♀	1/5	27-II-2009	TE
1015. *Hypoblemum* sp.	♀	3/4	27-II-2009	TE
1016. *Hypoblemum* sp.	♂	2/3	4-III-2008	PT
9617. *Hypoblemum albovittatum*	♂	Somerfield, Chch	5-II-2008	C.J. Vink
9799. *Hypoblemum albovittatum*	♂	Somerfield, Chch	1-III-2009	C.J. Vink
9800. *Hypoblemum albovittatum*	♀	Avondale, AK	8-III-2009	G. Hall
9801. *Hypoblemum albovittatum*	♀	Avondale, AK	8-III-2009	G. Hall
9802. *Hypoblemum albovittatum*	j.	Avondale, AK	8-III-2009	G. Hall
10033. *Hypoblemum albovittatum*	♂	Museum of NZ building, WN	5-V-2009	A. van Helden
1021. *Clubiona blesti*	♀	1/1	18-I-2008	TE
1022. *Clubiona blesti*	♀	1/3	20-II-2008	TE
1024. *Clubiona blesti*	♀	3/2	2-III-2008	TE
1025. *Clubiona blesti*	♀	2/2	14-XII-2008	TE
1026. *Clubiona blesti*	♂	3/5	2-III-2008	TE
1027. *Clubiona blesti*	♀	3/2	21-IX-2007	TE
1030. *Orepukia orophila*	♂	2/5	18-I-2008	TE
1031. *Orepukia orophila*	♀	3/4	2-III-2008	TE
1032. *Orepukia orophila*	♂	3/4	2-III-2008	TE
1033. *Orepukia orophila*	♀	4/3	3-III-2008	TE
1034. *Orepukia orophila*	♂	4/3	3-III-2008	TE
1036. *Orepukia orophila*	♂	5/2	3-III-2008	TE
1037. *Orepukia orophila*	♀	1/2	16-III-2008	TE
1038. *Orepukia orophila*	♀	1/3	16-III-2008	TE

Continued on next page

Table B.1 – continued from previous page

Sequence code and species	Sex	Collection site/plot	Collection date	Method or collector
1039. *Orepukia orophila*	♀	2/5	18-I-2008	TE
1040. *Orepukia poppelwelli*	♀	1/1	2-II-2008	TE
1049. *Orepukia poppelwelli*	♂	2/5	16-III-2008	TE
1045. *Orepukia poppelwelli*	♀	1/5	16-III-2008	TE
1041. *Orepukia poppelwelli*	♀	1/4	20-XII-2007	PT
1042. *Orepukia poppelwelli*	♀	1/1	2-II-2008	PT
1043. *Orepukia poppelwelli*	♀	2/2	2-II-2008	PT
1044. *Orepukia poppelwelli*	♀	1/4	4-III-2008	PT
1046. *Orepukia poppelwelli*	♀	1/5	4-III-2008	PT
1047. *Orepukia poppelwelli*	♂	2/1	4-III-2008	PT
1048. *Orepukia poppelwelli*	♀	3/2	2-III-2008	PT
1049. *Orepukia poppelwelli*	♂	2/4	16-III-2008	PT
9817. *Anoteropsis hilaris*	♀	5/2	28-II-2009	TE
1051. *Anoteropsis hilaris*	♂	2/4	21-XII-2007	PT
1052. *Anoteropsis hilaris*	♂	2/5	21-XII-2007	PT
1053. *Anoteropsis hilaris*	♂	3/4	21-XII-2007	PT
1054. *Anoteropsis hilaris*	♀	3/5	21-XII-2007	PT
1055. *Anoteropsis hilaris*	♂	4/2	23-XII-2007	PT
1056. *Anoteropsis hilaris*	♂	4/5	23-XII-2007	PT
1057. *Anoteropsis hilaris*	♀	5/3	3-III-2008	PT
1058. *Anoteropsis hilaris*	♂	5/3	3-III-2008	PT
1059. *Anoteropsis hilaris*	♀	3/3	2-II-2008	PT
1061. *Anoteropsis flavescens*	♂	5/5	17-III-2008	TE
1062. *Anoteropsis flavescens*	♀	2/2	14-XII-2008	TE
1063. *Anoteropsis flavescens*	♀	2/3	14-XII-2008	TE
1064. *Anoteropsis flavescens*	♀	5/5	17-III-2008	TE
1065. *Anoteropsis flavescens*	♀	4/1	15-XII-2008	TE
1066. *Anoteropsis flavescens*	♀	4/3	15-XII-2008	TE
1067. *Anoteropsis flavescens*	♀	1/3	27-II-2009	TE
1068. *Anoteropsis flavescens*	♂	1/3	27-II-2009	TE
1069. *Anoteropsis flavescens*	♂	3/1	27-II-2009	TE
1060. *Anoteropsis flavescens*	♀	3/4	27-II-2009	TE
9869. *Anoteropsis ralphi*	♀	Near Te Ranga	10-XII-1999	C.J. Vink
9870. *Anoteropsis ralphi*	♂	(43°55'S, 176°29'W)	9-XII-1999	
1082. *Laestrygones otagoensis*	♀	2/5	18-I-2008	TE
1083. *Laestrygones otagoensis*	♂	2/5	18-I-2008	TE
1084. *Laestrygones otagoensis*	♀	1/1	2-II-2008	TE

Continued on next page

Table B.1 – continued from previous page

Sequence code and species	Sex	Collection site/plot	Collection date	Method or collector
1085. *Laestrygones otagoensis*	♂	3/2	18-I-2008	TE
1086. *Laestrygones otagoensis*	♂	4/3	6-I-2008	TE
1087. *Laestrygones otagoensis*	♀	4/1	19-I-2008	TE
1088. *Laestrygones otagoensis*	♀	5/1	19-I-2008	TE
1089. *Laestrygones otagoensis*	♀	5/3	6-I-2008	TE
1091. *Laestrygones* sp.	♂	4/5	15-XII-2008	TE
1092. *Laestrygones* sp.	♀	1/4	22-I-2009	TE
1093. *Laestrygones* sp.	♀	2/4	27-II-2009	TE
1094. *Laestrygones* sp.	♀	1/1	14-XII-2008	TE

Table B.2: GenBank accession number and species name, family and references of sequences included in the analyses.

Sequence code (GenBank accession number and species)	Family	Reference
AF327993.1 *Marpissa pikei*	Salticidae	Hedin and Maddison, 2001
AY297417.1 *Trite planiceps*	Salticidae	Maddison and Hedin, 2003b
AY297419.1 *Castianeira* sp.	Clubionidae	Maddison and Hedin, 2003b
DQ127501.1 *Clubiona bishopi*	Clubionidae	Barrett and Hebert, 2005
EU979472.1 *Novalena intermedia*	Agelenidae	Paquin and Vink, 2009
DQ628619.1 *Neoramia janus*	Agelenidae	Spagna and Gillespie, 2008
EF584476.1 *Schizocosa rovneri*	Lycosidae	Hebets and Vink, 2007
AY059993.1 *Artoria separate*	Lycosidae	Vink et al., 2003
FJ607558.1 *Badumna longiqua*	Desidae	Blackledge et al., 2009
DQ628622.1 *Tengella radiate*	Tengellidae	Spagna and Gillespie, 2008
FJ607563.1 *Erigone dentosa*	Linyphiidae	Blackledge et al., 2009
AY297420.1 *Cesonia* sp.	Gnaphosidae	Maddison and Hedin, 2003b
DQ174370.1 *Misumenops dalmasi*	Thomisidae	Garb and Gillespie, 2006
FJ607562.1 *Dolomedes tenebrosus*	Pisauridae	Blackledge et al., 2009
FJ607559.1 *Callobius* sp.	Amaurobiidae	Blackledge et al., 2009

Table B.3: Models selected for each analysed taxa (maximum likelihood and Bayesian analyses).

Family	Species	Model with only congeneric species	Model with non congeneric species
Salticidae	*Hypoblemum* sp. *Hypoblemum albovittatum* *Marpissa pikei* *Trite planiceps*	GTR + Γ	GTR + Γ
Desidae	*Laestrygones otagoensis* *Laestrygones* sp. *Badumna longiqua*	TIM1 + Γ	GTR+ Γ
Agelenidae	*Orepukia orophila* *Orepukia poppelwelli* *Novalena intermedia* *Neoramia janus*	ML analysis: TrN + Γ Bayesian analysis: 1^{st} codon position: F81 2^{nd} codon position: F81 3^{rd} codon position: TIM1	GTR + Γ
Lycosidae	*Anoteropsis hilaris* *Anoteropsis flavescens* *Anoteropsis ralphi* *Arteria separate* *Schizocosa rovneri*	TIM1 + Γ	TIM3 + Γ
All of the above (GMYC model)		-	GTR+ I+ Γ

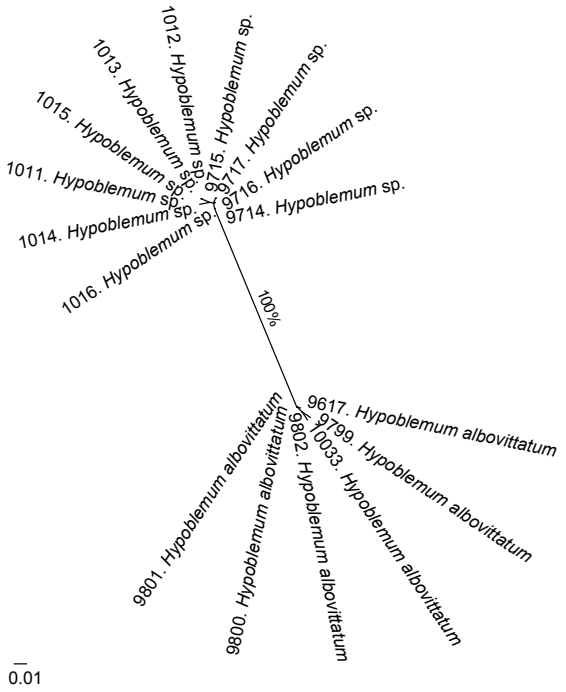

Figure B.2: Unrooted maximum likelihood tree of *Hypoblemum* sp. and *H. albovittatum*. Values above branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

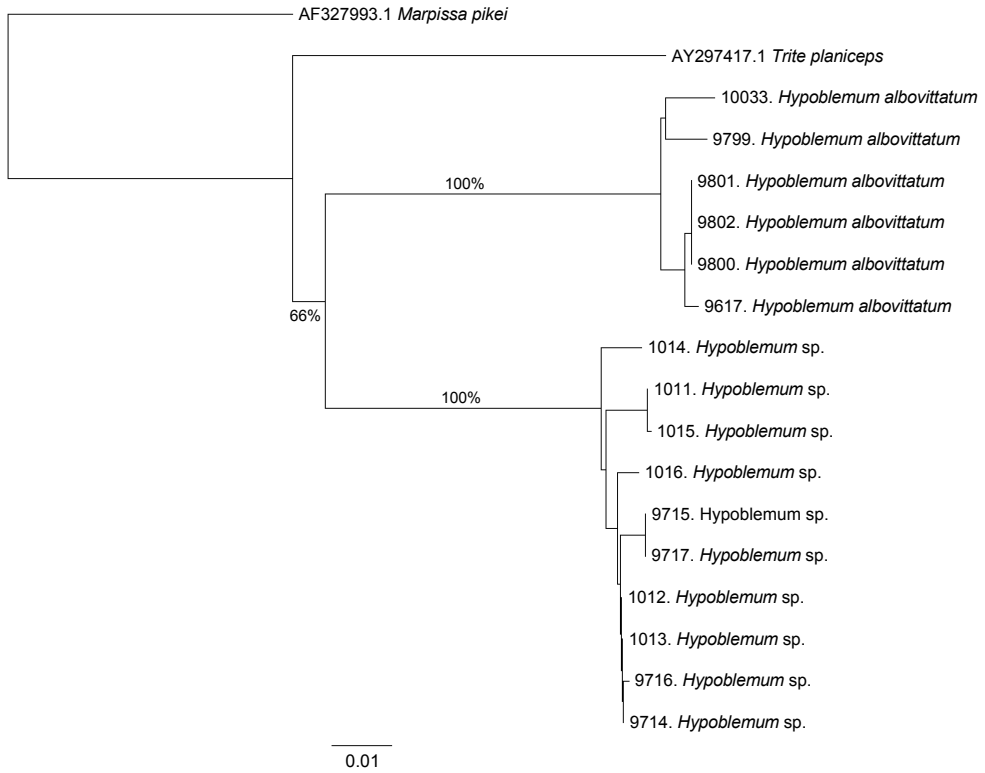

Figure B.3: Neighbour joining tree of *Hypoblemum* sp. and *H. albovittatum* specimens with outgroup sequences obtained from GenBank. Values next to branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

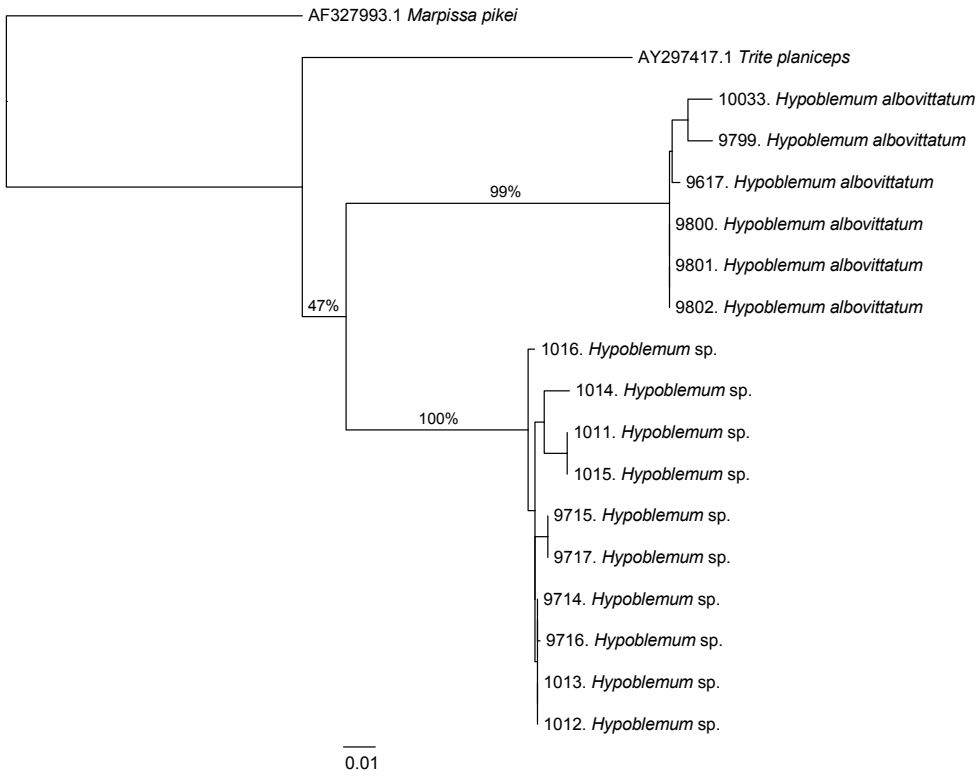

Figure B.4: Maximum likelihood tree of *Hypoblemum* sp. and *H. albovittatum* specimens with outgroup sequences obtained from GenBank. Values above branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

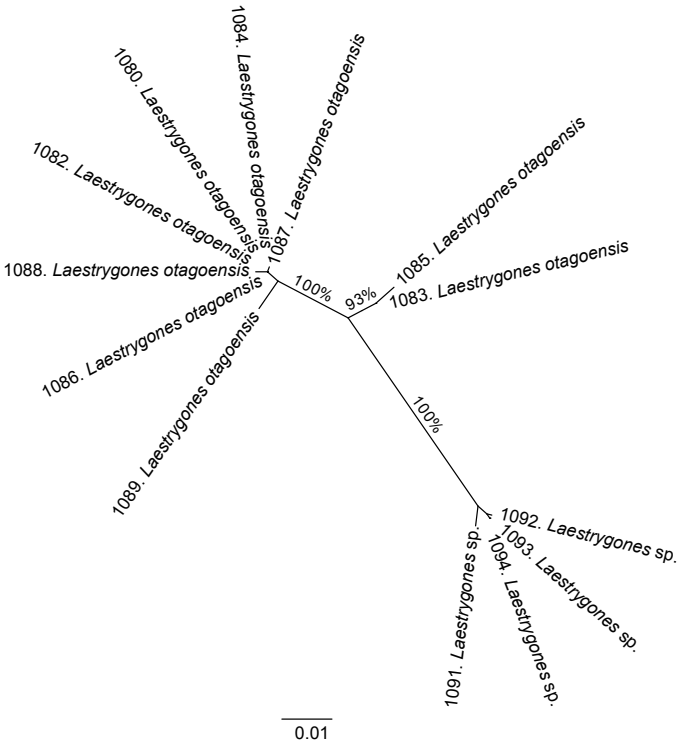

Figure B.5: Unrooted maximum likelihood tree of *Laestrygones* sp. and *L. otagoensis* specimens. Values above branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

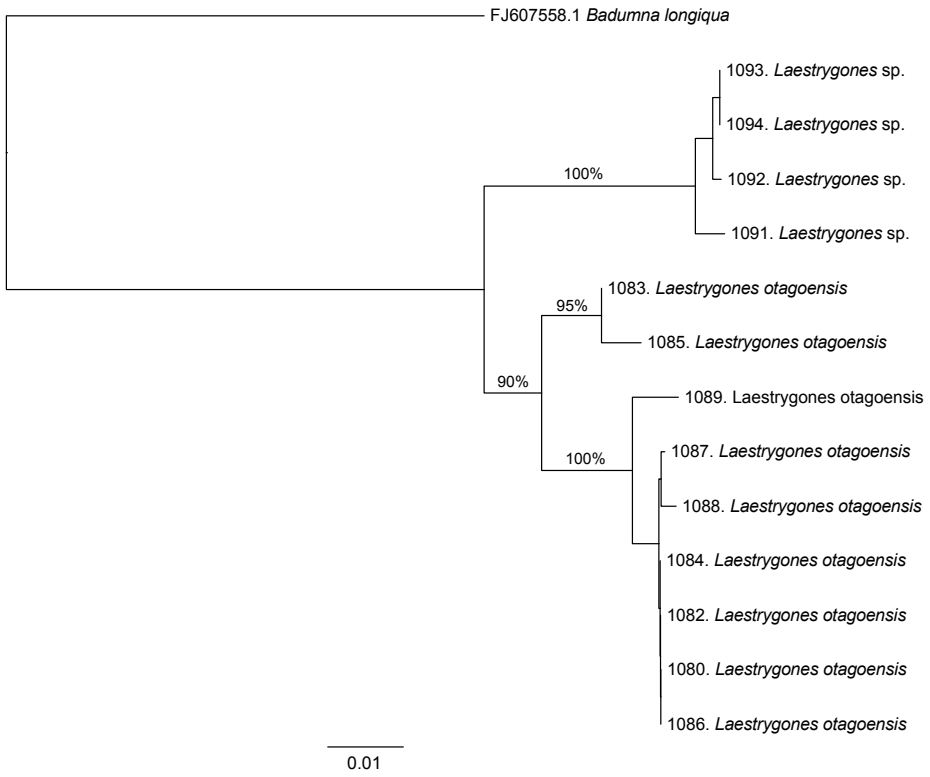

Figure B.6: Neighbour joining tree of *Laestrygones* sp. and *L. otagoensis* specimens with outgroup sequences obtained from GenBank. Values above branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

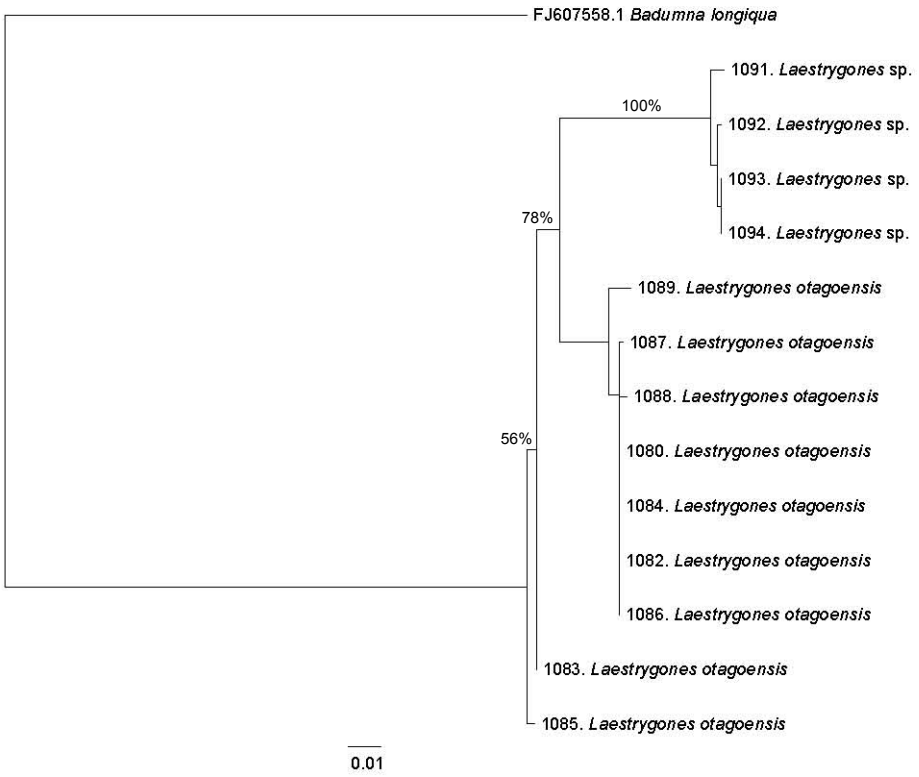

Figure B.7: Maximum likelihood tree of *Laestrygones* sp. and *L. otagoensis* specimens with outgroup sequences obtained from GenBank. Values above branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

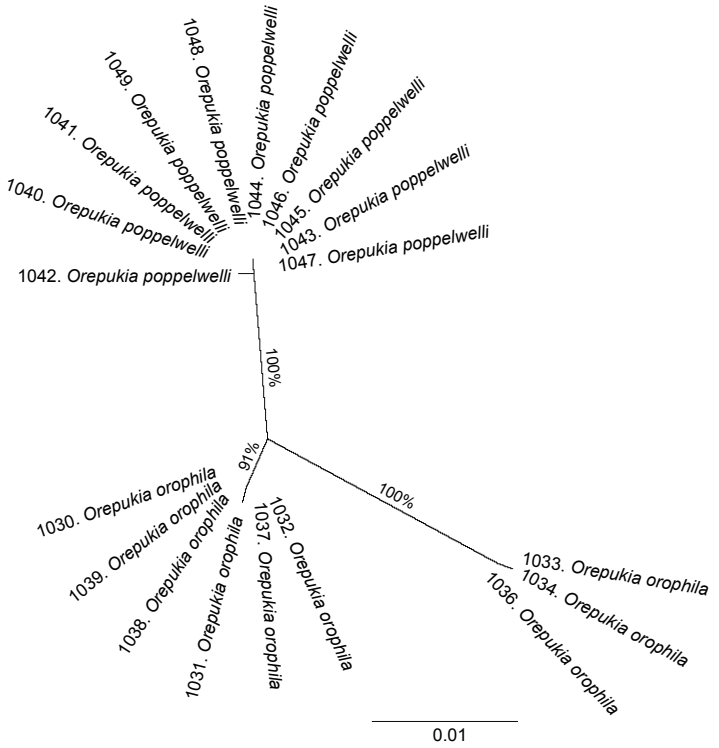

Figure B.8: Unrooted maximum likelihood tree of *O. orophila* and *O. poppelwelli* specimens. Values above branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

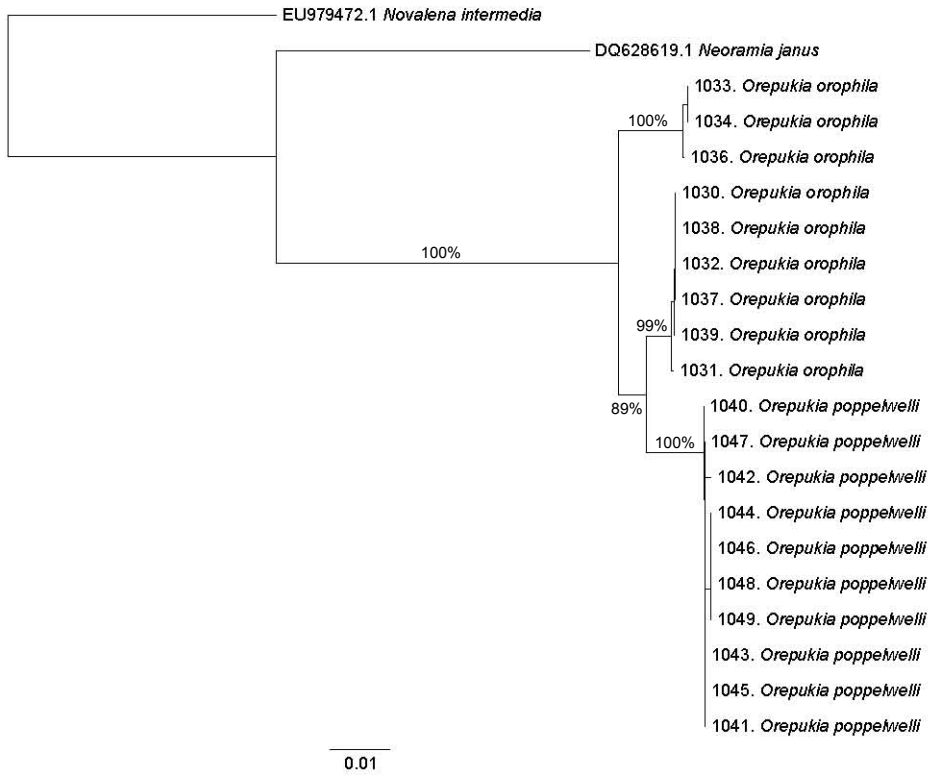

Figure B.9: Neighbour joining tree of *O. orophila* and *O. poppelwelli* specimens with outgroup sequences obtained from GenBank. Values next to branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

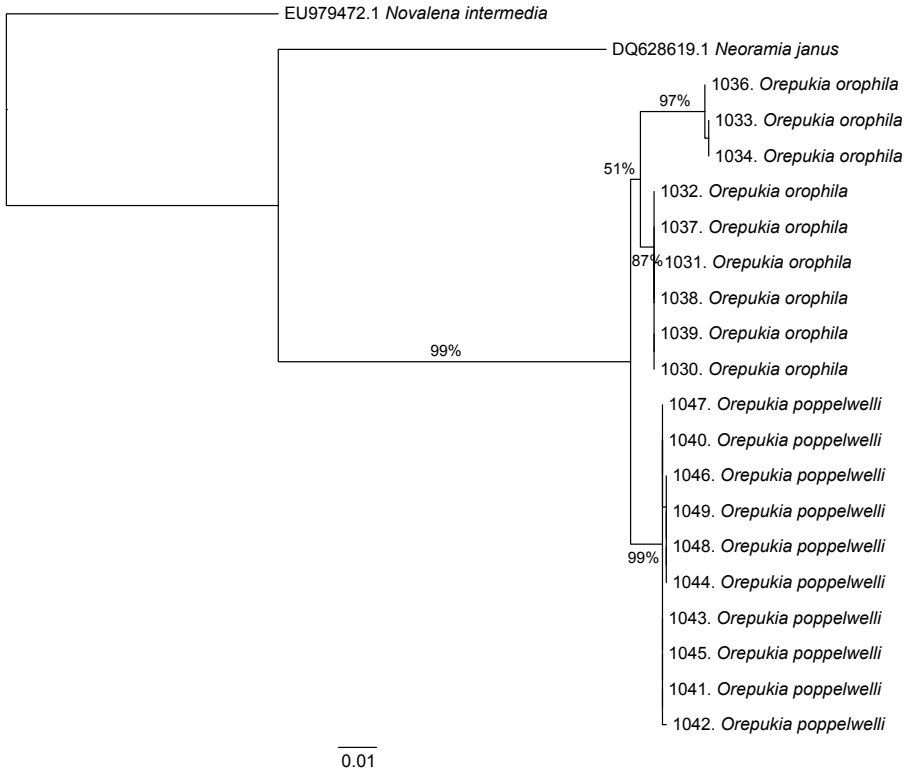

Figure B.10: Maximum likelihood tree of *O. orophila* and *O. poppelwelli* specimens with outgroup sequences obtained from GenBank. Values next to branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

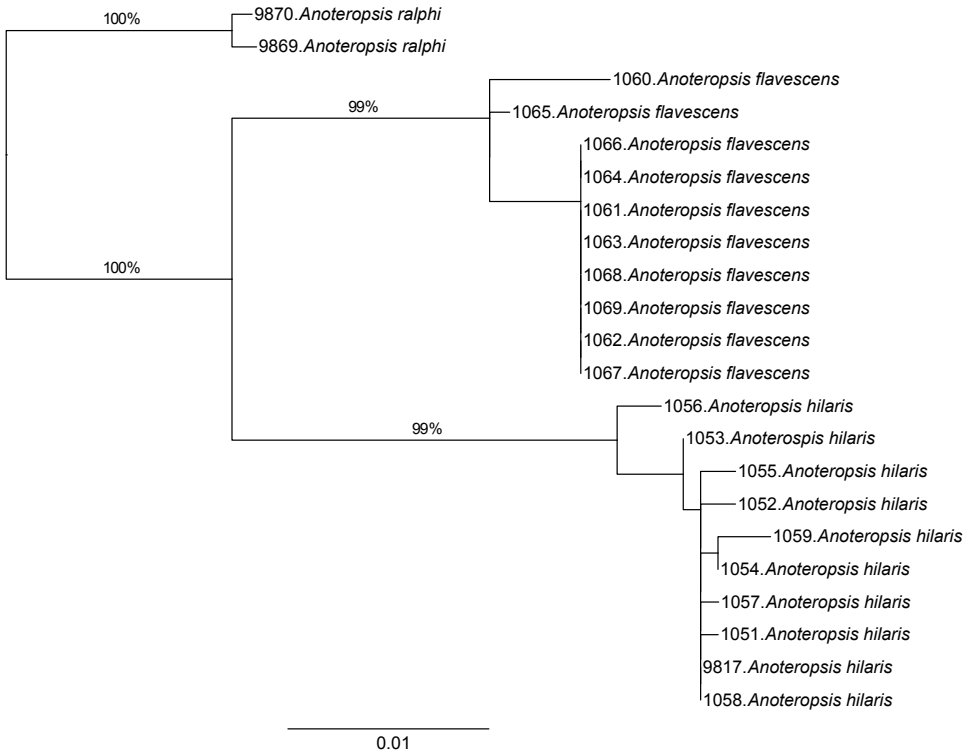

Figure B.11: Maximum likelihood tree of *A. hilaris*, *A. flavescens* and the outgroup *A. Ralphi* specimens. Values above branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

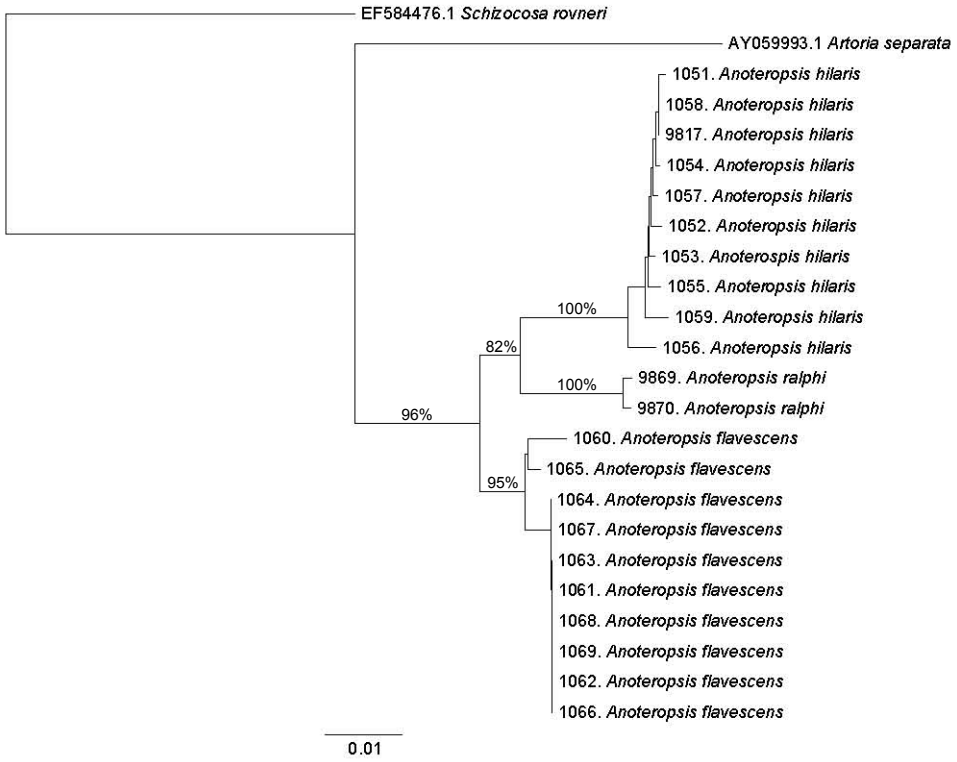

Figure B.12: Neighbour joining tree of the *A. hilaris* and *A. flavescens* specimens, the outgroup *A. Ralphi* and additional outgroup sequences obtained from GenBank. Values above branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

Figure B.13: Maximum likelihood tree of the *A. hilaris* and *A. flavescens* specimens, the outgroup *A. Ralphi* and additional outgroup sequences obtained from GenBank. Values next to branches are bootstrap support values. Only values of branches considered relevant for the analyses are shown.

Appendix C

Te Papanui Conservation Park: Spider and plant species

Table C.1: List of spider species collected in the study area.

		Sampling method	
		Turf	Pitfall
	Guild	extraction	trapping
Agelenidae			
Mahura rufula Forster & Wilton, 1973	Sheet web builder	X	X
Neoramia matua Forster & Wilton, 1973	Sheet web builder	X	X
Orepukia orophila Forster & Wilton, 1973	Sheet web builder	X	X
Orepukia poppelwelli Forster & Wilton, 1973	Sheet web builder	X	X
Amaurobiidae			
Pakeha maxima Forster & Wilton, 1973	Sheet web builder	X	X
Amphinectidae			
Aorangia sp.	Sheet web builder	X	X
Mamoea rufa (Berl&, 1931)	Runner	X	X
Clubionidae			
Clubiona blesti Forster, 1979	Runner	X	-
Corinnidae			
Supunna picta (L. Koch, 1873)	Runner	X	-
Cycloctenidae			
Toxopsiella lawrencei Forster, 1964	Runner	X	X
Desidae			
Laestrygones sp.	Runner	X	-
Laestrygones otagoensis Forster, 1970	Runner	X	X
Gnaphosidae			

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Table C.1 – continued from previous page

	Guild	Turf extraction	Pitfall trapping
Anzacia gemmea (Dalmas, 1917)	Runner	X	-
Nauhea tapa Forster, 1979	Runner	X	-
Hahniidae			
Kapanga sp.	Sheet web builder	X	X
Rinawa cantuaria Forster, 1970	Sheet web builder	X	X
Alistra sp.	Sheet web builder	X	X
Huttoniidae			
Huttoniidae sp.		X	X
Linyphiidae			
Araeoncus humilis (Blackwall, 1841)	Aerial web builder	X	-
Diplocephalus cristatus (Blackwall, 1833)	Aerial web builder	X	-
Diploplecta duplex Millidge, 1988	Aerial web builder	X	X
Dunedinia pullata Millidge, 1988	Aerial web builder	X	-
Erigone wiltoni Locket, 1973	Aerial web builder	X	-
Hyperafroneta obscura Blest, 1979	Aerial web builder	X	X
Laetesia minor Millidge, 1988	Aerial web builder	X	X
Laetesia trispathulata (Urquhart, 1886)	Aerial web builder	X	X
Metafroneta minima Blest, 1979	Aerial web builder	X	X
Haplinis inexacta (Blest, 1979)	Aerial web builder	X	-
Parafroneta minuta Blest, 1979	Aerial web builder	X	X
Protoerigone otagoa Blest, 1979	Aerial web builder	X	-
Tenuiphantes tenuis (Blackwall, 1852)	Aerial web builder	-	X
Lycosidae			
Anoteropsis flavescens L. Koch, 1878	Runners	X	X
Anoteropsis hilaris (L. Koch, 1877)	Runners	X	X
Micropholcommatidae			
Micropholcommatidae sp.1	Sheet web builder	X	-
Micropholcommatidae sp.2	Sheet web builder	X	X
Mysmenidae			
Mysmenidae sp.1	Aerial web builder	X	-
Trogloneta sp.	Aerial web builder	X	X
Orsolobidae			
Orsolobidae sp. (unknown)	Runner	X	-
Orsolobidae sp.1	Runner	X	X
Orsolobidae sp.2	Runner	X	X
Orsolobidae sp.3	Runner	X	X
Orsolobidae sp.4	Runner	X	X

Continued on next page

Table C.1 – continued from previous page

	Guild	Turf extraction	Pitfall trapping
Orsolobidae sp.5	Runner	X	X
Salticidae			
Salticidae sp.2	Stalker	X	-
Salticidae sp.3	Stalker	X	-
Salticidae sp.4	Stalker	X	-
Salticidae sp.5	Stalker	X	-
Salticidae sp.7	Stalker	X	-
Hypoblemum sp.	Stalker	X	X
Stiphidiidae			
Cambridgea arboricola (Urquhart, 1891)	Sheet web builder	X	X
Cambridgea secunda Forster & Wilton, 1973	Sheet web builder	X	X
Synotaxidae			
Pahora sp.	Aerial web builder	X	-
Theridiidae			
Theridiidae sp.1	Aerial web builder	X	X
Cryptachaea sp.	Aerial web builder	X	-
Total number of species/morphospecies		54	33

Table C.2: List of plant species reccorded in the study area.

Species
Acaena caesiglauca (Bitter) Bergmans (1939)
Acaena fissistipula Bitter (1911)
Aciphylla hectorii Buchanan (1881) [1882]
Agrostis capillaries L. (1753)
Anisotome aromatica Hook.f.
Anisotome sp.
Anthoxanthum odoratum L. (1753)
Astelia linearis var.*novae-zel&iae* Skottsb. (1934)
Asteria nervosa Hook.f. (1853)
Blechnum penna-marina (Poir.) Kuhn (1868)
Cardamine sp.
Carpha alpine R.Br. (1810)
Celmisia gracilenta Hook.f. (1844)
Celmisia prorepens Petrie (1887)
Celmisia sessiliflora Hook.f. (1864)
Chionochloa rigida (Raoul) Zotov (1963)
Coprosma perpusilla Colenso (1889) [1890]
Coprosma petriei Cheeseman (1885) [1886]
Craspedia lanata (Hook.f.) Allan (1961)
Craspedia uniflora G.Forst. (1786)
Drosera arcturi Hook. (1834)
Epilobium sp.
Epilobium tenuipes Hook.f. (1852)
Euphrasia sp.
Gaultheria depressa Hook.f. (1847)
Gentiana bellidifolia Hook.f. (1844)
Gentiana grisebachii Hook.f. (1844)
Gentiana sp.
Geranium microphyllum Hook.f. (1844)
Geum leiospermum Petrie (1893) [1894]
Gingidia baxterae (J.W.Dawson) C.J.Webb
Hebe hectorii (Hook.f.) Cockayne & Allan (1926)
Hebe odora (Hook.f.) Cockayne (1929)
Helichrysum filicaule Hook.f. (1852)
Hieracium lepidulum (Stenstr.) Omang
Hieracium pilosella L.
Hydrocotyle novae-zeel&iae DC. (1830)

Continued on next page

Table C.2 – continued from previous page

Species
Hypochaeris radicata L.
Kelleria dieffenbachii (Hook.) Endl. (1847)
Leucopogon colensoi Hook.f. (1864)
Leucopogon fraseri A.Cunn. (1839)
Luzula rufa var. *albicomans* Edgar (1966)
Lycopodium fastigiatum R.Br. (1810)
Melicytus alpinus (Kirk) Garn.-Jones (1987)
Myosotis pygmaea Colenso (1883) [1884]
Myrsine nummularia Hook.f.
Oreobolus pectinatus Hook.f. (1844)
Oreomyrrhis colensoi Hook.f. var. *colensoi*
Oreomyrrhis rigida (Kirk) Allan ex Mathias & Constance
Pentachondra pumila (J.R.Forst. & G.Forst.) R.Br. (1810)
Pernettya macrostigma Colenso (1888) [1889]
Pimelea oreophila C.J.Burrows
Plantago novae-zel&iae L.B.Moore (1961)
Poa colensoi Hook.f. (1864)
Prasophyllum colensoi Hook.f. (1853)
Psychrophila novae-zel&iae (Hook.f.) W.A.Weber (1982)
Pterostylis sp.
Ranunculus enysii Kirk (1879) (1880)
Ranunculus reflexus Garn.-Jones (1987)
Raoulia subulata Hook.f. (1864)
Rytidosperma sp.
Schizeilema sp.
Scleranthus uniflorus P.A.Williamson (1956)
Uncinia divaricata Boott (1853)
Unknown sp.1
Unknown sp.2
Wahlenbergia albomarginata Hook. (1852)

Appendix D

Deep Stream: Spider species and treatment comparisons

Table D.1: List of spider species collected in the study area. Classification of species according to their origin is based on the literature used for specimen identification (see methods in chapter 5).

	Guild	Origin
Agelenidae		
Huka minuta Forster and Wilton, 1973	Sheet web builder	Endemic
Mahura rufula Forster and Wilton, 1973	Sheet web builder	Endemic
Amaurobiidae		
Poaka graminicola Forster and Wilton, 1973	Sheet web builder	Endemic
Amphinectidae		
Mamoea rufa (Berland, 1931)	Runner	Endemic
Maniho meridionalis Forster and Wilton, 1973	Sheet web builder	Endemic
Anapidae		
Anapidae sp.	Aerial web builder	Probably endemic
Zealanapis sp.	Aerial web builder	Endemic
Araneidae		
Eriophora pustulosa (Walckenaer, 1842)	Aerial web builder	Native
Clubionidae		
Clubiona blesti Forster, 1979	Runner	Endemic
Clubiona clima Forster, 1979	Runner	Endemic
Corinnidae		
Supunna picta (L. Koch, 1873)	Runner	Exotic
Desidae		
Badumna longinqua (L.Koch,1867)	Runner	Exotic

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Table D.1 – continued from previous page

	Guild	Origin
Gasparia rustica Forster, 1970	Runner	Endemic
Hapona sp.	Runner	Endemic
Laestrygones otagoensis Forster, 1970	Runner	Endemic
Dictynidae		
Arangina sp.	Aerial web builder	Endemic
Gnaphosidae	Runner	
Anzacia gemmea (Dalmas, 1917)	Runner	Native
Nauhea tapa Forster, 1979	Runner	Endemic
Zelanda obtuse (Forster, 1979)	Runner	Endemic
Hahniidae		
Alistra sp.	Sheet web builder	Endemic
Rinawa cantuaria Forster, 1970	Sheet web builder	Endemic
Huttoniidae		
Huttoniidae sp.	Runner	Endemic
Linyphiidae		
Araeoncus humilis (Blackwall, 1841)	Aerial web builder	Exotic
Diplocephalus cristatus (Blackwall, 1833)	Aerial web builder	Exotic
Diploplecta duplex Millidge, 1988	Aerial web builder	Endemic
Dunedinia pullata Millidge, 1988	Aerial web builder	Endemic
Erigone wiltoni Locket, 1973	Aerial web builder	Exotic
Haplinis fucatinia (Urquhart,1894)	Aerial web builder	Endemic
Haplinis inexacta (Blest, 1979)	Aerial web builder	Endemic
Haplinis subdola (O.Pickard-Cambridge, 1879)	Aerial web builder	Endemic
Hyperafroneta obscura Blest, 1979	Aerial web builder	Endemic
Laetesia minor Millidge, 1988	Aerial web builder	Endemic
Laetesia trispathulata (Urquhart, 1886)	Aerial web builder	Endemic
Maorineta tumida Millidge, 1988	Aerial web builder	Endemic
Parafroneta minuta Blest, 1979	Aerial web builder	Endemic
Protoerigone otagoa Blest, 1979	Aerial web builder	Endemic
Tenuiphantes tenuis (Blackwall, 1852)	Aerial web builder	Exotic
Lycosidae		
Allotrochosina schauinslandi (Simon, 1899)	Runner	Endemic
Anoteropsis adumbrate (Urquhart, 1887)	Runner	Endemic
Anoteropsis flavescens L. Koch, 1878	Runner	Endemic
Anoteropsis hilaris (L. Koch, 1877)	Runner	Endemic
Micropholcommatidae		
Micropholcommatidae sp.1	Sheet web builder	Probably endemic
Micropholcommatidae sp.2	Sheet web builder	Probably endemic

Continued on next page

Table D.1 – continued from previous page

	Guild	Origin
Micropholcommatidae sp.3	Sheet web builder	Probably endemic
Micropholcommatidae sp.4	Sheet web builder	Probably endemic
Mysmenidae		
Mysmenidae sp.1	Aerial web builder	Probably endemic
Mysmenidae sp.2	Aerial web builder	Probably endemic
Orsolobidae		
Orsolobidae sp.1	Runner	Probably endemic
Orsolobidae sp.2	Runner	Probably endemic
Orsolobidae sp.5	Runner	Probably endemic
Orsolobidae sp.6	Runner	Probably endemic
Salticidae		
Hypoblemum sp.	Stalker	Unknown
Salticidae sp.2	Stalker	Unknown
Salticidae sp.3	Stalker	Unknown
Salticidae sp.4	Stalker	Unknown
Salticidae sp.6	Stalker	Unknown
Salticidae sp.7	Stalker	Unknown
Stiphidiidae		
Cambridgea secunda Forster and Wilton, 1973	Sheet web builder	Endemic
Tetragnathidae		
Tetragnathidae sp.	Aerial web builder	Unknown
Theridiidae		
Cryptachaea blattea (Urquhart, 1886)	Aerial web builder	Exotic
Cryptachaea sp.	Aerial web builder	Unknown
Theridiidae sp.3	Aerial web builder	Unknown
Theridiidae sp.4	Aerial web builder	Unknown
Steatoda grossa (C. L. Koch, 1838)	Aerial web builder	Exotic
Steatoda lepida (O. Pickard-Cambridge, 1879)	Aerial web builder	Endemic
Thomisidae		
Sidymella angularis (Urquhart, 1885)	Ambusher	Endemic

Table D.2: Pairwise comparisons between control, spring and summer burn treatments for the modelled response variables. Only significantly different ($p<0.05$ and $p<0.01$) comparisons are shown.

Variable	Year	Compared treatments	d.f.	t statistic	Actual p value	Adjusted p value
Number of individuals ($\log_{10}$)						
	2001	Control–Summer burn	36	-3.3535	<0.001	<0.01
	2002	Control–Spring burn	36	2.9192	<0.01	<0.05
	2003	Control–Spring burn	36	2.4808	<0.01	<0.05
Number of species ($\log_{10}$)						
	2000	Control–Summer	36	2.7805	<0.01	<0.05
	2002	Control–Spring burn	36	6.6921	<0.001	<0.01
	2002	Spring burn–Summer burn	36	2.6321	<0.01	<0.05
	2003	Control–Summer burn	36	6.8018	<0.001	<0.01
	2004	Control–Summer burn	36	3.9729	<0.001	<0.01
	2005	Control–Spring burn	36	4.0864	<0.001	<0.01
Number of families ($\log_{10}$)						
	2000	Control–Spring burn	36	3.2190	<0.01	<0.01
	2002	Control–Spring burn	36	8.0011	<0.001	<0.01
	2003	Control–Summer burn	36	8.3052	<0.001	<0.01
	2004	Control–Summer burn	36	5.6283	<0.001	<0.01
	2005	Control–Spring burn	36	5.4585	<0.001	<0.01
ACE ($\log_{10}$)						
	2002	Control–Spring burn	36	3.8962	<0.001	<0.01
	2003	Control–Summer burn	36	3.3866	<0.001	<0.01
Shannon's H''(sqrt)						
	2002	Control–Summer burn	36	3.5338	<0.001	<0.01

Continued on next page

Table D.2 —continued from previous page

Variable	Year	Compared treatments	d.f.	t statistic	Actual p value	Adjusted p value
Guilds						
Runners						
(transformed percentage)	2005	Control–Spring burn	36	3.8678	<0.001	<0.01
Aerial web builders						
(transformed percentage)	2003	Control–Summer burn	36	-3.4767	<0.01	<0.01
	2004	Control–Summer burn	36	-3.4645	<0.001	<0.01
	2005	Control–Summer burn	36	-2.8626	<0.01	<0.05
Exotic spiders						
(transformed percentage)						
	2002	Control–Summer burn	36	-3.0028	<0.01	<0.05
	2003	Control–Spring burn	36	-5.1391	<0.001	<0.01
	2004	Control–Summer burn	36	-4.6508	<0.001	<0.01
	2005	Control–Spring burn	36	-4.046	<0.001	<0.01
Families						
Linyphiidae ($\log_{10}$)						
	2001	Control–Spring burn	36	-3.4040	<0.001	<0.01
	2005	Control–Summer burn	36	-2.8260	<0.01	<0.05
	2005	Control–Spring burn	36	-4.4865	<0.001	<0.01
Gnaphosidae ($\log_{10}$)						
	2001	Control–Summer burn	36	-3.0909	<0.01	<0.01
	2003	Control–Spring burn	36	2.8837	<0.01	<0.05
Species						
Diplocephalus cristatus						
($\log_{10}$ (x+0.5))	2003	Control–Summer burn	36	-2.6667	<0.01	<0.05
	2004	Control–Summer burn	36	-4.1096	<0.001	<0.01
	2005	Control–Spring burn	36	-2.6601	<0.01	<0.05

Appendix E

Published research I

The following research study was conducted and the paper published as an additional project during the Ph.D..

CSIRO PUBLISHING
www.publish.csiro.au/journals/is *Invertebrate Systematics*, 2008, **22**, 589–604

Species status and conservation issues of New Zealand's endemic *Latrodectus* spider species (Araneae : Theridiidae)

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Abstract. New Zealand has two endemic widow spiders, *Latrodectus katipo* Powell, 1871 and *L. atritus* Urquhart, 1890. Both species face many conservation threats and are actively managed. The species status of the *Latrodectus* spiders of New Zealand was assessed using molecular (COI, ITS1, ITS2) and morphological methods and with cross-breeding experiments. *Latrodectus katipo* and *L. atritus* were not found to be reciprocally monophyletic for any of the gene regions or morphological traits. Other than colour, which is variable, there were no morphological characters that separated the two species, which cross-bred in the laboratory and produced fertile eggsacs. Colour variation is clinal over latitude and correlates significantly with mean annual temperature. We conclude that *L. atritus* is a junior synonym of *L. katipo*. An example of introgression from the Australian species *L. hasseltii* Thorell, 1870 was also detected and its conservation implications are discussed.

Additional keywords: conservation genetics, cytochrome oxidase subunit 1 (COI), DNA, internal transcribed spacer regions (ITS), intraspecific variation, *Latrodectus atritus*, *Latrodectus hasselti,Latrodectus hasseltii*, *Latrodectus katipo*, phylogenetics, taxonomy.

Introduction

Widow spider species in the genus *Latrodectus* Walckenaer, 1805 are found in xeric habitats throughout the world and, probably owing to their potent neurotoxic venom, do not usually inspire thoughts of conservation. However, New Zealand's endemic *Latrodectus* species face a number of conservation threats, such as habitat loss and modification (Patrick 2002) and competition from exotic spider species (Hann 1990). Currently, two *Latrodectus* species are considered endemic to New Zealand: *L. katipo* Powell, 1870 and *L. atritus* Urquhart, 1890. *Latrodectus katipo* has a degree of cultural significance in New Zealand as one of only a few endemic invertebrates given a name by Māori (*katipō* = night stinger) and its image has been used on a postage stamp. The New Zealand Department of Conservation (DoC) has classified *L. katipo* and *L. atritus* as in 'Serious Decline' (Hitchmough *et al*. 2007).

A problematic issue in the conservation of New Zealand's *Latrodectus* species is whether there are indeed two species, which has management implications for DoC. Because DoC conservation priorities and decisions are often based on species (Towns and Williams 1993), the criteria used to assign a species particular conservation status should be based on the best available taxonomic evidence (see Paquin *et al*. 2008). As a widespread and medically important genus, *Latrodectus* has been subject to a great degree of taxonomic scrutiny over the years (e.g. Levi 1959, 1983) and this has had implications on the status of the New Zealand species. *Latrodectus katipo* was described in 1871 by Powell and the specimen he described and illustrated had a red median stripe (Powell 1871). Powell noted that the species was 'tolerably numerous' in the North Island but rare in the South Island. Urquhart (1890) described *Latrodectus atritus* as a variety of *L. katipo* and Parrott (1948), who believed *L. katipo* to be a junior synonym of *L. hasseltii* Thorell, 1870 (the Australian redback), listed *L. atritus* as a subspecies of *L. hasseltii*. Levi (1959) considered *L. atritus* (along with *L. katipo* and *L. hasseltii*) a junior synonym of *L. mactans* (Fabricius, 1775) (the North American black widow). However, Forster and Forster (1973) rejected Levi's (1959) synonymy and retained the name *L. katipo*. Forster (1975) elevated *L. atritus* to species and noted that *L. katipo* and *L. atritus* differed in colouration, which has been used to separate some *Latrodectus* species (McCrone and Levi 1964; Lotz 1994). *Latrodectus katipo* females have a red median stripe on the dorsal surface of the abdomen (Powell 1871) whereas the dorsal surface of *L. atritus* is completely black (Urquhart 1890; McCutcheon 1976; Forster and Kingsford 1983; Forster and Forster 1999). *Latrodectus atritus* males are considered to have paler colours and patterns than those of *L. katipo* males (Forster and Kingsford 1983). McCutcheon (1976) noted colour variations in *L. atritus*, which can have a red stripe on the dorsal surface of the abdomen of the adult female, although not as bright as that of *L. katipo*. Forster and Kingsford (1983) also noted differences between the species in the time it took for spiderlings to emerge from the eggsac. However, the authors labelled their study as preliminary and noted that there is variability in colour, patterning and all aspects of the life cycle in most species of *Latrodectus*. Forster and Forster (1999: 175) stated that "laboratory studies show that they [*L. katipo* and *L. atritus*] do not generally crossmate but when sometimes they do, the eggs are infertile" but no data were included to support this.

Usually, spider species differ in the structures of their genitalia (the male pedipalp and the female epigynum) (Eberhard 1986; Coddington and Levi 1991; Huber 2004); however, discrimination between *Latrodectus* species based on genitalia has been problematic (Levi 1983). Griffiths *et al*. (2005) found no difference between the genitalia of the two New Zealand species, but they did not examine internal female genitalia. This is also the case for *L. katipo* and the Australian *L. hasseltii* (Parrott 1948; Levi 1959). However, there is a reliable morphological character that separates females of *L. katipo* from females of *L. hasseltii*: the abdomen of *L. katipo* is covered in dense short fine setae whereas *L. hasseltii* is comparatively sparsely covered with both long fine setae and stouter short setae (Kavale 1986; Forster and Forster 1999). In addition, *L. hasseltii* females are usually larger than *L. katipo* females (Kavale 1986; Forster and Forster 1999) whereas *L. katipo* males are larger than *L. hasseltii* males (Forster 1995). *Latrodectus hasseltii* also performs a stereotyped behaviour of sexual cannibalism (Forster 1992; Andrade 1996). Laboratory studies have shown that *L. hasseltii* females will not mate with *L. katipo* males (Kavale 1986; Forster 1992), which may be due to *L. katipo* males being heavier (Forster 1995). However, *L. hasseltii* males will mate with *L. katipo* females (Kavale 1986; Forster 1992, 1995). Phylogenetic studies including New Zealand and Australian *Latrodectus* species show that *L. katipo* and *L. hasseltii* are closely related but separate, monophyletic species (Garb *et al*. 2004; Griffiths *et al*. 2005).

Griffiths *et al*. (2005) explored the intraspecific and interspecific relationships of *L. katipo* and *L. atritus* using the mitochondrial gene region NADH dehydrogenase subunit 1 (ND1). They found no support for the separation of the two species but because of their limited sample size they were unable to conclusively comment as to whether *L. atritus* was a junior synonym of *L. katipo*. To properly ascertain the species status of New Zealand *Latrodectus* spp. Griffiths *et al*. (2005) recommended a larger sample of spiders and the use of a faster evolving section of the mitochondrial genome, such as cytochrome oxidase subunit 1 (COI). In many spiders, ND1 is more divergent than COI (e.g. Hedin and Wood 2002; Vink and Paterson 2003); however, the divergence between *L. katipo* and *L. hasseltii* in COI (Garb *et al*. 2004) was more than three times that of ND1 (Griffiths *et al*. 2005).

Both New Zealand species inhabit coastal dune systems and commonly build webs in low growing plants and driftwood or flotsam. *Latrodectus katipo* inhabits coastal dunes in the South Island and in the North Island to approximately 38° South (Griffiths 2001). *Latrodectus atritus* inhabits coastal dunes in the North Island from the far north to approximately 39°15′ South (Forster and Forster 1999; Sutton *et al*. 2006). There is an overlap in the species' distributions between these two latitudes on both coasts of the North Island (McCutcheon 1976, 1992; Forster and Forster 1999; Sutton *et al*. 2006) (see Fig. 1). This overlap also correlates with the transition area where mean annual temperature along the coast changes from 13.1–14°C to 14.1–15°C (National Institute of Water and Atmospheric Research NZ 2004).

In this paper, we examine the species status of the two New Zealand *Latrodectus* species using molecular and morphological criteria. Moritz (1994) suggested that reciprocal monophyly in mitochondrial DNA (mtDNA) alleles and significant divergence in nuclear loci can be used as criteria for evolutionarily significant units (ESUs). Therefore sequence data were used to establish whether the mtDNA and/or the nuclear DNA lineages of *L. katipo* and *L. atritus* were reciprocally monophyletic. We examined morphological features to ascertain if there were any differences between the two species and compared them with the sister-species, *L. hasseltii*. We also cross-bred specimens of *L. katipo* and *L. atritus* to test the claim that the two species do not cross-breed (Forster and Forster 1999) and to further investigate whether there were any biological barriers between the two species.

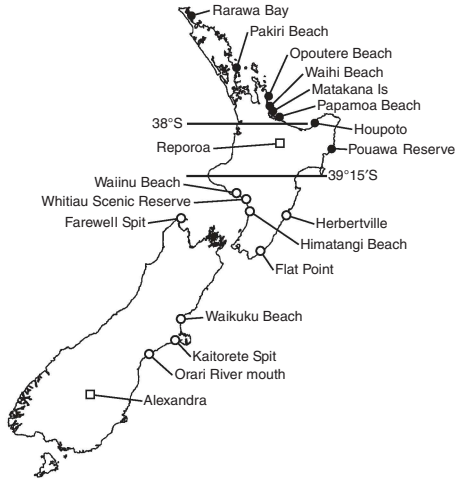

Fig. 1. Distribution of sites sampled for molecular analyses of *Latrodectus* spiders in New Zealand: ○ *L. katipo*; ● *L. atritus*; □ *L. hasseltii*. 38°S is marked on the map to show the northern limit of *L. katipo* and 39°15′S indicates the southern limit of *L. atritus*.

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Materials and methods

Molecular analyses

Twenty specimens of *L. katipo*, 14 specimens of *L. atritus* and seven specimens of *L. hasseltii* were used for the molecular analyses (see Table 1). *Latrodectus katipo* and *L. atritus* were hand-collected from throughout their range in New Zealand (see Fig. 1). *Latrodectus hasseltii* was chosen as the outgroup for the molecular analyses because it is the closely related sister-species to *L. katipo* and *L. atritus* (Garb *et al*. 2004; Griffiths *et al*. 2005; J.A. Miller, pers. comm.). Specimens were collected from the west and east of Australia as well as New Zealand as a preliminary exploration of *L. hasseltii* genetic diversity. Voucher specimens were deposited at the Museum of New Zealand Te Papa Tongarewa, New Zealand (MONZ) and Entomology Research Museum, Lincoln University, New Zealand (LUNZ).

For our molecular criteria, we selected COI and a section of the nuclear genome spanning the two ribosomal internal transcribed spacer regions (ITS1 and ITS2), all of the nuclear ribosomal RNA subunit 5.8S and small fragments of the flanking 18S and 28S. COI is one of the fastest evolving mtDNA genes and has been used to examine genetic differences between spider species and populations (Hedin and Wood 2002; Vink and Paterson 2003; Croucher *et al*. 2004; Paquin and Hedin 2004; Ayoub *et al*. 2005; Bond *et al*. 2006), including *Latrodectus* (Garb *et al*. 2004). The nuclear ribosomal RNA subunit (5.8S, 18S and 28S) sequences were unlikely to vary between closely related *Latrodectus* species and are typically used for deeper level phylogenetic studies (e.g., Arnedo *et al*. 2004); however, ITS1 and ITS2 have been used at the species and population level in spiders (Hedin 1997; Arnedo and Gillespie 2006; Chang *et al*. 2007), including a study that examined the taxonomic status of two North American *Latrodectus* species (Zhang *et al*. 2004).

DNA was extracted using DNeasy Tissue Kits (QIAGEN, Valencia, CA, USA) from two or three legs from each specimen (except for *La*6 and *Lh*4 where the cephalothorax was used). Initially, the primers used to amplify the COI fragments were LCO-1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) (Folmer *et al*. 1994) plus C1-N-2776-spider (5′-GGATAAT CAGAATANCGNCGAGG-3′) (Vink *et al*. 2005). However, these primers occasionally amplified two segments of DNA, the 1261-bp COI sequence segment and a 676-bp mitochondrial pseudogene, which contained stop codons and deletions. To avoid the amplification of pseudogenes the primer pair LCO1490 plus C1-N-2568 (5′-GCTACAACATAATA AGTATCATG-3′) (Hedin and Maddison 2001) was used to amplify a 1054-bp COI segment. The primer pair used to amplify ITS1 and ITS2 (~1040 bp) of the nuclear rDNA internal transcribed spacer regions were CAS18sF1 (5′-TACACACCGCCCGTCGCTA CTA-3′) and CAS28sB1d (5′-TTCTTTTCCTCCSCTTAY TRATATGCTTAA-3′) (Ji *et al*. 2003). *Ex Taq* DNA Polymerase (Takara Bio Inc., Otsu, Shiga, Japan) was used for most of the PCR amplifications but in a few cases Platinum *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA, USA) was used instead. PCR amplification was performed in a Mastercycler (Eppendorf, Hamburg, Germany) thermocycler with a cycling profile of 40 cycles of 94°C denaturation (30 s), 48°C annealing (40 s), 72°C extension (1 min) with an initial denaturation of 3 min and a final extension of 5 min. For the amplification of ITS1 and ITS2, the annealing temperature was 55°C. Excess primers and salts were removed from the resulting double-stranded DNA by using polyethylene glycol (PEG)/ NaCl precipitation. A Perfectprep Gel Cleanup kit (Eppendorf) was used to purify PCR products where pseudogenes were produced and in two instances where soil fungi were also amplified (*Fusarium* sp. and *Mucor* sp.) along with ITS1 and ITS2. For specimen *La*8, amplification of ITS1 and ITS2 was not possible owing to contamination by a *Mucor* sp. fungus that preferentially annealed to the primers. Fungal contaminants were identified by BLAST searching their sequences (Altschul *et al*. 1997).

Purified PCR fragments were sequenced in both directions at the Microchemical Core Facility (San Diego State University, San Diego, CA, USA), the Waikato DNA Sequencing Facility (University of Waikato, Hamilton, New Zealand) or the Allan Wilson Centre Genome Service (Massey University, Palmerston North, New Zealand). Sequence data were deposited in GenBank (www.ncbi.nlm.nih.gov/Genbank/index.html; verified November 2008); see Table 1 for accession numbers.

Sequences were edited using Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequencher was also used for the alignment of COI sequences because there was no evidence of insertions/deletions or stop codons and alignment was straight forward. ITS1 and ITS2 sequences were aligned using default alignment settings in Clustal X (Thompson *et al*. 1997). Uncorrected pairwise distances were calculated using PAUP* version 4.0b10 (Swofford 2002). Partitioned Bayesian analyses, based on the methods of Brandley *et al*. (2005), were implemented in MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) to estimate the COI phylogenetic tree topology. MrModeltest version 2.2 (Nylander 2005) implemented in PAUP* version 4.0b10 (Swofford 2002) was used to select the model parameters. Within MrModeltest, the Akaike Information Criterion was used for model selection (Posada and Buckley 2004). The COI data were partitioned by codon, using the models Hasegawa–Kishino–Yano (HKY; Hasegawa *et al*. 1985) for the 1st codon positions, F81 (Felsenstein 1981) for the 2nd codon positions and general time reversible (GTR; Lanave *et al*. 1984; Tavaré 1986; Rodríguez *et al*. 1990; Yang *et al*. 1994) for the 3rd codon positions. Bayesian analyses were conducted by running two simultaneous, completely independent analyses each with four heated chains, sampling every 1000th tree. The analyses were run until the average standard deviation of split frequencies had dropped below 0.001, which indicated that the two tree samples had converged. This occurred after 807 273 000 generations.

MrBayes was used to construct majority rule consensus trees, discarding the first 25% of trees generated as burn-in. TreeView 1.6.6 (Page 1996) was used to view and save trees in graphic format. ITS1 and ITS2 sequences were not analysed phylogenetically as there was only one nucleotide that varied in New Zealand *Latrodectus* specimens and no variation between *L. hasseltii* specimens.

Abbreviations for New Zealand locations and institutions

The two-letter location codes used in the text follow Crosby *et al*. (1998).

AMNZ Auckland Museum, New Zealand
CMNZ Canterbury Museum, New Zealand

Table 1. Specimens used for molecular analyses

The two letters in italics in the specimen codes denote the species; *La* = *Latrodectus atritus*, *Lk* = *Latrodectus katipo*, *Lh* = *Latrodectus hasseltii*

Specimen code	Sex	Location	COI haplotype	ITS haplotype	GenBank accession nos.	Cross-breeding information
*La*1	Female	NZ, GB, Pouawa Reserve (38°37′S, 178°11′E), 4.vii.2004, JWG	18	A	EF121006, EF121038	
*La*2	Female	NZ, CL, Waihi Beach (37°24′S, 175°57′E), 13.vi.2004, JWG	10	A	EF121007, EF121039	
*La*3	Female	NZ, CL, Waihi Beach (37°24′S, 175°57′E), 13.vi.2004, JWG	11	A	EF121008, EF121040	
*La*4	Female	NZ, ND, Rarawa Bay (34°44′S, 173°05′E) , 1999, JWG	6	A	EF121009, EF121041	
*La*5	Female	NZ, CL, Opoutere Beach (37°06′S, 175°53′E), 1999, JWG	12	A	EF121010, EF121042	
*La*6	Female	NZ, GB, Houpoto (37°58′S, 177°33′E), 1999, JWG	13	A	EF121011, EF121043	
*La*7	Male	NZ, BP, Matakana Is (37°31′S, 176°02′E), 16.xi.2004, M.E. Sutton	14	A	EF121012, EF121044	
*La*8	Male	NZ, BP, Matakana Is (37°31′S, 176°02′E), 16.xi.2004, M.E. Sutton	15	Unknown	EF121013	
*La*9	Juvenile	NZ, BP, Papamoa Beach (37°42′S, 176°17′E), 17.ii.2005, B.R. Christensen	16	A	EF121014, EF121045	
*La*10	Subadult female	NZ, AK, Pakiri Beach (36°15′S, 174°43′E), 20.i.2006, J.W. Early	17	A	EF121015, EF121046	
*La*11	Female	NZ, AK, Pakiri Beach (36°15′S, 174°43′E), 20.i.2006, J.W. Early	17	A	EF121016, EF121047	
*La*12	Female	NZ, BP, Papamoa Beach (37°43′S, 176°16′E), 19.ii.2007, B.R. Christensen	16	A	EU309678, EU309679	mated with Lk15
*La*13	Male	NZ, BP, Papamoa Beach (37°45′S, 176°25′E), 1.vi.2007, JM-O	14	A	EU305448, EU305456	mated with Lk16
*La*14	Female	NZ, BP, Papamoa Beach (37°45′S, 176°25′E), 1.vi.2007, JM-O	15	A	EU305449, EU305457	mated with Lk18
*La*15	Male	NZ, BP, Papamoa Beach (37°45′S, 176°25′E), 1.vi.2007, JM-O	Unknown	Unknown		mated with Lk17
*Lk*1	Female	NZ, WI, Waiinu Beach (39°52′S, 174°44′E), 24.vii.2004, DoC staff	1	B	EF121017, EF121048	
*Lk*2	Female	NZ, WI, Himatangi Beach (40°23′S, 175°14′E), 4.vii.2004, DoC staff	2	AB	EF121018, EF121049	
*Lk*3	Subadult female	NZ, WI, Whitiau Scenic Reserve (40°02′S, 175°06′E), 24.vii.2004, DoC staff	3	B	EF121019, EF121050	
*Lk*4	Female	NZ, WA, Herbertville (40°29′S, 176°37′E), 1999, JWG	4	B	EF121020, EF121051	
*Lk*5	Female	NZ, WA, Herbertville (40°29′S, 176°37′E), 1999, JWG	5	AB	EF121021, EF121052	
*Lk*6	Female	NZ, NN, Farewell Spit (40°30′S, 172°48′E), 1999, JWG	6	AB	EF121022, EF121053	
*Lk*7	Female	NZ, NN, Farewell Spit (40°30′S, 172°48′E), 1999, JWG	6	B	EF121023, EF121054	
*Lk*8	Female	NZ, WA, Flat Point (41°28′S, 175°37′E), 1999, JWG	7	A	EF121024, EF121055	
*Lk*9	Female	NZ, WA, Flat Point (41°28′S, 175°37′E), 1999, JWG	7	AB	EF121025, EF121056	
*Lk*10	Female	NZ, WA, Flat Point (41°28′S, 175°37′E), 1999, JWG	7	A	EF121026, EF121057	
*Lk*11	Male	NZ, MC, Kaitorete Spit (43°49′S, 172°40′E), 10.iii.2005, A.M. Evans	8	A	EF121027, EF121058	
*Lk*12	Female	NZ, MC, Kaitorete Spit (43°49′S, 172°40′E), 10.iii.2005, A.M. Evans	8	A	EF121028, EF121059	
*Lk*13	Female	NZ, NC, Waikuku Beach (43°17′S, 172°43′E), 1.iv.2006, CIV	8	AB	EF121029, EF121060	
*Lk*14	Female	NZ, NC, Waikuku Beach (43°17′S, 172°43′E), 1.iv.2006, CIV	6	AB	EF121030, EF121061	
*Lk*15	Male	NZ, MC, Kaitorete Spit (43°49′S, 172°40′E), 12.iv.2007, CIV, JM-O, AMP	7	AB	EU305450, EU305458	mated with La12
*Lk*16	Female	NZ, MC, Kaitorete Spit (43°49′S, 172°40′E), 12.iv.2007, CIV, JM-O, AMP	7	A	EU305451, EU305459	mated with La13
*Lk*17	Female	NZ, MC, Kaitorete Spit (43°49′S, 172°40′E), 12.iv.2007, CIV, JM-O, AMP	7	AB	EU305452, EU305460	mated with La15
*Lk*18	Male	NZ, MC, Kaitorete Spit (43°49′S, 172°40′E), 11.vii.2007, S. Brown	9	A	EU305453, EU305461	mated with La14
*Lk*19	Female	NZ, SC, near Orari River mouth (44°14′S, 171°25′E), 6.ix.2007, CIV, W.G. Chinn, D. Anderson	7	AB	EU305454, EU305462	
*Lk*20	Male	NZ, SC, near Orari River mouth (44°14′S, 171°25′E), 6.ix.2007, CIV, W.G. Chinn, D. Anderson	7	AB	EU305455, EU305463	
*Lh*1	Subadult female	Aus, Western Australia, Wembley (31°56′S, 115°48′E), 10.xi.2004, M. Ruitenberg	19	C	EF121031, EF121062	
*Lh*2	Subadult female	Aus, Western Australia, Carine (31°51′S, 115°46′E), 22.xi.2004, L. Timmins	20	C	EF121032, EF121063	
*Lh*3	Female	Aus, Western Australia, Nedlands (31°58′S, 115°48′E), 15.xi.2004, V.W. Framenau	21	C	EF121033, EF121064	
*Lh*4	Female	Aus, Queensland, Brisbane (27°29′S, 153°01′E), 1999, R.J. Raven	21	C	EF121034, EF121065	
*Lh*5	Subadult male	NZ, BP, Reporoa (38°26′S, 176°20′E), 2.iii.2005	22	C	EF121035, EF121066, EF121069	
*Lh*6	Female	NZ, CO, Alexandra (45°15′S, 169°24′E), 1.xii.2005, P. Batson	23	C	EF121036, EF121067, EF121070	
*Lh*7	Female	Aus, Western Australia, Nedlands (31°58′S, 115°48′E), 15.xi.2004, V.W. Framenau	22	C	EF121037, EF121068	

DoC Department of Conservation, New Zealand
LUNZ Entomology Research Museum, Lincoln University, New Zealand
MONZ Museum of New Zealand Te Papa Tongarewa, New Zealand
AgR Biosecurity Group, AgResearch, New Zealand.

Morphological analyses

For our morphological criteria we examined whether there were any consistent morphological differences between the two putative species other than the abdominal colour pattern. Museum specimens from MONZ, AMNZ and LUNZ were examined for possible morphological differences. Two sets of measurements were made. Levy and Amitai (1983) and Kavale (1986) both used a patella–tibial index to differentiate between *Latrodectus* species. As defined by Kavale (1986), this is the total length of the patella and tibia of leg I divided by the carapace length from the clypeal apex to the posterior notch. Kavale (1986) presented measurements for 50 *L. katipo* females and 67 *L. hasseltii* specimens. We measured the patella–tibial index for 21 female specimens of *L. atritus*. A set of standard general morphological measurements of 12 specimens of *L. katipo* and 13 specimens of *L. atritus* (Table 6) were also made. In this case, a standard carapace length (clypeal apex to hindmost point of posterior margin) was used instead of the shorter clypeal apex–posterior notch measurement used for the patella–tibial index. Measurements were made using a Leica MZ6 microscope and Leica DC30 camera in conjunction with IM50 software (Leica Microsystems, Wetzlar, Germany). Female abdominal setae, male pedipalps and external and internal female genitalia were compared between *L. katipo*, *L. atritus* and *L. hasseltii* specimens. Terminology of the male pedipalpal structures follows Agnarsson *et al*. (2007). We also examined the types of *L. katipo* var. *atritus*, *Theridium melanozantha* Urquhart, 1887 and *T. zebrinia* Urquhart, 1890, which are all held at CMNZ (Nicholls *et al*. 2000). The type(s) of *L. katipo* are lost (Levi 1959).

Cross-breeding

We investigated whether *L. katipo* and *L. atritus* could cross-breed by coupling specimens from two locations within the species' ranges: Papamoa Beach and Kaitorete Spit (see Fig. 1 and Table 1). Females (*La*14, *Lk*16, *Lk*17) were collected as subadults and were kept in the laboratory until they moulted. This ensured the females were unmated before the cross-breeding experiments. An adult female *L. atritus* (*La*12) was collected from Papamoa Beach and kept for 50 days. Despite a plentiful supply of food, she did not produce an eggsac and was assumed not to have been previously mated. The unmated adult females (*La*12, *La*14, *Lk*16, *Lk*17) were placed in a cabinet at 25°C with a light regime of 14-h light and 10-h darkness to simulate summer conditions when breeding occurs. A male was introduced into the cage with a female of the other species (see Table 1) and observed. The females were observed daily to note whether any eggsacs were produced and, if so, whether any spiderlings emerged. All specimens from the cross-breeding experiment were sequenced except for *La*15 (*L. atritus* male), which was lost (probably consumed by the *L. katipo* female, *Lk*17). Collection details of the specimens are listed in Table 1.

Correlation between colouration and temperature

The correlation between abdominal colouration and mean annual temperature was tested using a Mantel test (Mantel 1967). All *L. atritus* and *L. katipo* specimens examined in the study (both for molecular and morphological analyses) were classified based on colouration and coded as either 1 (red abdominal stripe present – *L. katipo*) or 0 (red abdominal stripe absent – *L. atritus*) in a triangular model matrix following Legendre and Legendre (1998: 557). A second matrix coding for environmental conditions used the mean annual temperature of the specimen collection locality. For each locality record, the value for mean annual temperature was calculated from 10 years (1 Jan 1997–31 Dec 2006) of Virtual Climate Station data (see Tait *et al*. 2006) from the National Institute of Water and Atmospheric Research, New Zealand (www.niwa.cri.nz; verified November 2008). A distance matrix measuring all pairwise associations between mean temperatures was generated using Euclidian distances (D01 in Legendre and Legendre 1998). The Mantel test was performed in the R Package Version 4.0 (Casgrain and Legendre 2000) with a total of 10 000 permutations. This method is equivalent to a nonparametric multivariate analysis of variance (MANOVA, see Legendre and Legendre 1998).

Results

Molecular analyses

Eighteen COI haplotypes occurred among the 34 specimens of New Zealand *Latrodectus*, one of which (*La*1) had a *L. hasseltii* COI sequence (see Table 1). However, specimen *La*1 had an ITS sequence that matched New Zealand species of *Latrodectus* and also had a dense covering of short, fine setae on the body, which is characteristic of *L. katipo*. Pairwise distances between COI sequences of *L. katipo, L. atritus* and *L. hasseltii* are shown in Table 2. Only two nucleotide changes were nonsynonymous, one of which was found in specimen *La*3 and the other in specimen *Lk*18. These specimens were PCR amplified and sequenced an additional time to confirm that the nonsynonymous change was not due to polymerase error.

Of the 1025 nucleotides of ITS data, five were variable and there were no insertions or deletions. One nucleotide varied in New Zealand *Latrodectus* species. This nucleotide was towards the 3′ end of ITS2 and was thymine (haplotype A), adenine (haplotype B) or a mix of the two nucleotides (haplotype AB) (see Table 1). It is possible that all New Zealand *Latrodectus* specimens have two copies of ITS2 and the sequences with either adenine or thymine are just a result of random PCR amplification of one copy over the other. In all specimens of *L. hasseltii* that we sampled, this nucleotide was always thymine. There was no variation in the ITS sequences of any of the *L. hasseltii* we sampled. Pairwise distances between ITS sequences of *L. katipo, L. atritus* and *L. hasseltii* are shown in Table 3. As expected, there was no variation in the 18S, 5.8S and 28S sequences.

The phylogenetic analysis of the COI data (Fig. 2) showed that, except for *La*1, *L. hasseltii* was monophyletic. There was no evidence for the reciprocal monophyly of *L. atritus*. There was also no relationship between the COI phylogeny for *L. katipo* and *L. atritus* and the ITS haplotypes.

Table 2. Uncorrected distance matrix for cytochrome *c* oxidase I (COI)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	*Lh*1																						
2	*Lh*2	0.002																					
3	*Lh*3, *Lh*4	0.001	0.001																				
4	*Lh*5, *Lh*7	0.002	0.002	0.001																			
5	*Lh*6	0.004	0.004	0.003	0.004																		
6	*La*1	0.002	0.002	0.001	0.002	0.004																	
7	*La*2	0.031	0.031	0.030	0.031	0.029	0.029																
8	*La*3	0.030	0.030	0.029	0.030	0.028	0.028	0.003															
9	*La*5	0.030	0.030	0.029	0.030	0.028	0.028	0.003	0.002														
10	*La*6	0.031	0.031	0.030	0.031	0.029	0.029	0.006	0.005	0.005													
11	*La*7, *La*13	0.029	0.029	0.028	0.029	0.027	0.027	0.002	0.001	0.001	0.004												
12	*La*8, *La*14	0.028	0.028	0.027	0.028	0.026	0.026	0.003	0.002	0.002	0.005	0.001											
13	*La*9, *La*12	0.031	0.031	0.030	0.031	0.029	0.029	0.006	0.005	0.005	0.002	0.004	0.005										
14	*La*10, *La*11	0.029	0.029	0.028	0.029	0.027	0.027	0.003	0.002	0.002	0.005	0.001	0.002	0.005									
15	*Lk*1	0.031	0.031	0.030	0.031	0.029	0.029	0.006	0.005	0.005	0.004	0.004	0.005	0.004	0.005								
16	*Lk*2	0.030	0.030	0.029	0.030	0.028	0.028	0.005	0.004	0.004	0.003	0.003	0.004	0.003	0.004	0.001							
17	*Lk*3	0.029	0.029	0.028	0.029	0.027	0.027	0.006	0.005	0.005	0.004	0.004	0.005	0.004	0.005	0.004	0.003						
18	*Lk*4	0.032	0.032	0.031	0.032	0.030	0.030	0.007	0.006	0.006	0.001	0.005	0.006	0.003	0.006	0.005	0.004	0.005					
19	*Lk*5	0.030	0.030	0.029	0.030	0.028	0.028	0.011	0.010	0.010	0.007	0.009	0.008	0.009	0.010	0.009	0.008	0.009	0.008				
20	*Lk*6, *Lk*7, *Lk*14, *La*4	0.029	0.029	0.028	0.029	0.027	0.027	0.004	0.003	0.003	0.002	0.002	0.003	0.002	0.003	0.002	0.001	0.002	0.003	0.007			
21	*Lk*8, *Lk*9, *Lk*10, *Lk*15, *Lk*16, *Lk*17, *Lk*19, *Lk*20	0.029	0.029	0.028	0.029	0.027	0.027	0.010	0.009	0.009	0.006	0.008	0.007	0.008	0.009	0.008	0.007	0.008	0.007	0.001	0.006		
22	*Lk*11, *Lk*12, *Lk*13	0.030	0.030	0.029	0.030	0.028	0.028	0.005	0.004	0.004	0.003	0.003	0.004	0.003	0.004	0.003	0.002	0.001	0.004	0.008	0.001	0.007	
23	*Lk*18	0.030	0.030	0.029	0.030	0.028	0.028	0.011	0.010	0.010	0.007	0.009	0.008	0.009	0.010	0.009	0.008	0.009	0.008	0.002	0.007	0.001	0.008

Table 3. Uncorrected distance matrix for ITS1 and ITS2

		1	2	3
1	*Lh*1, *Lh*2, *Lh*3, *Lh*4, *Lh*5, *Lh*6, *Lh*7			
2	*Lk*1, *Lk*3, *Lk*4, *Lk*7	0.005		
3	*Lk*2, *Lk*5, *Lk*6, *Lk*9, *Lk*13, *Lk*14, *Lk*15, *Lk*17, *Lk*19, *Lk*20	0.004	0.000	
4	*La*1, *La*2, *La*3, *La*4, *La*5, *La*6, *La*7, *La*9, *La*10, *La*11, *La*12, *La*13, *La*14, *Lk*8, *Lk*10, *Lk*11, *Lk*12, *Lk*16, *Lk*18	0.004	0.001	0.000

Of the 1025 nucleotides of ITS data, five were variable and there were no insertions or deletions. One nucleotide varied in New Zealand *Latrodectus* species. This nucleotide was towards the 3′ end of ITS2 and was thymine (haplotype A), adenine (haplotype B) or a mix of the two nucleotides (haplotype AB) (see Table 1). It is possible that all New Zealand *Latrodectus* specimens have two copies of ITS2 and the sequences with either adenine or thymine are just a result of random PCR amplification of one copy over the other. In all specimens of *L. hasseltii* that we sampled, this nucleotide was always thymine. There was no variation in the ITS sequences of any of the *L. hasseltii* we sampled. Pairwise distances between ITS sequences of *L. katipo, L. atritus* and *L. hasseltii* are shown in Table 3. As expected, there was no variation in the 18S, 5.8S and 28S sequences.

The phylogenetic analysis of the COI data (Fig. 2) showed that, except for *La*1, *L. hasseltii* was monophyletic. There was no evidence for the reciprocal monophyly of *L. atritus*. There was also no relationship between the COI phylogeny for *L. katipo* and *L. atritus* and the ITS haplotypes.

Morphological analyses

The patella–tibial index for *L. atritus* was 1.58 (see Table 4), which is identical to Kavale's (1986) result for *L. katipo*. The patella–tibial index for *L. hasseltii* is 1.86 (Kavale 1986). The means and standard deviations for each morphological measurement are given in Table 5. In females, no mean for any measurement for either species was outside the standard deviation for the other. In males, the means for *L. atritus* were within the standard deviations for *L. katipo* but the reverse was not always true, probably because of the greater variation in *L. katipo* size observed in this small sample; however, all standard deviations overlapped. The abdominal setae on females of *L. katipo* and *L. atritus* varied in density between specimens (Fig. 3*E*, *J*), but there was no consistent difference in the type and arrangement of the setae between the two species. We also observed variations in the male pedipalps and female genitalia of *L. katipo* and *L. atritus* (see Fig. 3*A–D*, *F–I*) but there were no consistent differences between the species. There were, however, consistent morphological differences between *L. hasseltii* and the New Zealand *Latrodectus* species: *L. katipo* and *L. atritus* have a triangular-shaped sclerotised area at the base of the embolus (Fig. 3*A*, *F*), which can be present or absent (Fig. 3*K*) in *L. hasseltii*; the edge of the theridioid tegular apophysis (TTA) is curled ventrally in *L. katipo* and *L. atritus* so that the knob-like projections on the dorsal surface of the TTA are visible at the anterior and ectal edges of the TTA when viewed ventrally (Fig. 3*B*, *G*); the knob-like projections are not visible in the ventral view of *L. hasseltii* (Fig. 3*L*); the rounded anterior end of the spermatheca in *L. hasseltii* is approximately one-half of the total length of the spermatheca (Fig. 3*N*) but only approximately one-third in *L. katipo* and *L. atritus* (Fig. 3*D*, *I*); *L. katipo* and *L. atritus* have a posteriorly directed hump at the centre of the anterior edge of the depression of the external genitalia (Fig. 3*C*, *H*); in *L. hasseltii* the posteriorly directed hump can be present, reduced or absent (Fig. 3*M*); the type and arrangement of abdominal setae differ (Fig. 3*E*, *J*, *O*).

The female holotype of *L. katipo* var. *atritus* was in reasonable condition and its external genitalia and setae on the abdomen matched those of other *L. atritus* and *L. katipo* species. The type

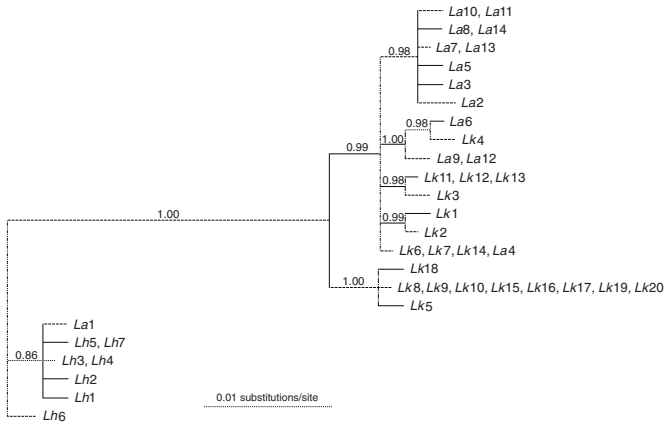

Fig. 2. Bayesian consensus tree based on cytochrome *c* oxidase I (COI) sequence data. Values above branches are posterior probabilities. Specimen codes are listed in Table 1.

specimens of *T. melanozantha* (six male syntypes) and *T. zebrinia* (one immature female holotype) were in very poor condition, but it was possible to confirm from abdominal markings that they were *L. katipo*. The specimens were likely to have been in good condition when Bryant (1933) and Levi (1959) examined them, but the types had probably degraded in the years after R.R. Forster left the CMNZ.

Cross-breeding

Mating was observed in all cross-breeding pairs. All females produced eggsacs between 8 and 13 days after mating. Mated females produced two or three eggsacs except *La*12, which produced eight eggsacs in total. Spiderlings emerged approximately 3 weeks after the first eggsac was produced. Spiderlings emerged from all eggsacs except those produced by *Lk*17. One female spiderling from *La*12 and three female spiderlings from *Lk*16 were reared through to adults; all females had a red median stripe on the dorsal surface of the abdomen during their early instars, but this was lost by the final moult in the female produced by the cross-breeding of *La*12 and *Lk*15 and the red stripe was reduced in the three females produced by the cross-breeding of *Lk*16 and *La*13. Voucher specimens are stored at LUNZ.

Correlation between colouration and temperature

Abdominal colouration is significantly correlated with mean annual temperature (correlation coefficient $R = 0.675$, $P = 0.0001$). *Latrodectus atritus* was found at localities where the mean annual temperature ranged from 13.64 to 16.23°C. Mean annual temperatures at *L. katipo* localities ranged from 11.24 to 13.85°C.

Discussion

Closely related *Latrodectus* species can be separated by low genetic divergence (Garb *et al*. 2004) and slight morphological differences (Kaston 1970; Lotz 1994). Therefore, criteria with which to separate *Latrodectus* species must be as fine as possible. We believe the criteria we tested would give two separate *Latrodectus* species the best possible chance to reveal themselves. *Latrodectus katipo* and *L. atritus* do not show reciprocal monophyly in mtDNA and the slight differences in the nuclear DNA we examined showed no relation to the two species. *Latrodectus atritus*, therefore, does not meet Moritz's (1994) suggested criteria for an ESU. Several authors have argued for a broader categorisation of ESUs than Moritz (1994) proposed (Crandall *et al*. 2000; Rader *et al*. 2005). Crandall *et al*. (2000) argued that genetic and ecological exchangeability be considered when considering population distinctiveness in conservation biology. Our data show no reason to reject the null hypothesis of genetic exchangeability and it is likely that there is gene flow between New Zealand populations. Further supporting the likelihood of gene flow is the fact that *L. katipo* and *L. atritus* disperse by ballooning. Griffiths (2001) simulated light wind in the laboratory; some early instars of *L. katipo* and *L. atritus* would ascend the stalks of marram grass (*Ammophila arenaria* (L.)) and release a long thread of silk from their spinnerets, which would create enough lift to carry the spiderling away. Ballooning in the right conditions could enable *L. katipo* and *L. atritus* to disperse over considerable distances and might explain why their distributions span numerous geographic barriers such as headlands, estuaries, rivers and areas of open sea (<30 km). The dispersal capabilities of *L. katipo* and *L. atritus*, an overlap of their distributions between 38° and 39°15′ South and very similar ecological niches (Griffiths 2001), suggest that there is ecological exchangeability.

Rader *et al*. (2005) suggested that life-history traits be considered to determine ecological exchangeability. However, this does not seem appropriate for *Latrodectus* because many life history traits show much intraspecific plasticity. Kaston (1970) noted intraspecific variations in several aspects of the life cycles of the North American widow species *L. mactans*, *L. hesperus* Chamberlin & Ivie, 1935 and *L. variolus* Walckenaer, 1837. It is likely that the differences in eggsac maturation time reported for *L. katipo* and *L. atritus* (Forster and Kingsford 1983; Forster and Forster 1999) fall within intraspecific variation. The findings of Forster and Kingsford (1983) are tenuous at best for two reasons. First, the *L. katipo* specimens were taken from the southern limit of the species' range (Karitane Beach, Otago) and, therefore, any differences observed could be a cold-adapted population difference. Second, the eggsacs tested all originated from just five females (four *L. katipo* and one *L. atritus*), so the data could easily be skewed by low sample size and may well have been within the range found for the species.

Cross-breeding experiments have been conducted on other *Latrodectus* species and separate species generally do not produce fertile eggs (Kaston 1970; Schmidt 1990); however, Forster (1992) successfully cross-bred *L. hasseltii* males with *L. katipo* females. Kasumovic and Andrade (2004) found that mating between *L. hesperus* from disparate populations resulted in non-viable eggs, but there were morphological differences between the two populations they tested (Kasumovic and Andrade 2004) and there is some evidence for genetically distinct populations (J.A. Miller, pers. comm.). Cross-breeding experiments in *Latrodectus* are certainly not a definitive method for delimiting species but should be considered with other morphological and molecular evidence. Although our experiment was limited and we did not test whether F1 spiders were fertile, it does refute the statement of Forster and Forster (1999) that cross-breeding between *L. katipo* and *L. atritus* seldom occurs and produces infertile eggs. The failure of the three eggsacs produced by *Lk*17 is not surprising because *Latrodectus* eggs often do not develop to the hatching stage (Kaston 1970). It is interesting to note that the female produced by the cross-breeding of *La*12 and *Lk*15 lost her red dorsal abdominal stripe by her final moult and the red stripe was reduced in the three females produced by the cross-breeding of *Lk*16 and *La*13.

Abdominal colouration is known to vary intraspecifically in North American and African *Latrodectus* species (McCrone and Levi 1964; Kaston 1970; Lotz 1994), the Australian *L. hasseltii* (Raven and Gallon 1987) and New Zealand *Latrodectus* species (McCutcheon 1976; Griffiths 2001; Sutton *et al*. 2006). The southern limits of *L. atritus* on both the east and west coasts of the North Island are almost identical (39°10′ and 39°17′ respectively – Forster and Forster 1999) and the northern limits of *L. katipo* are also very similar (37°45′ on the east coast and 38°04′ on the west – Griffiths 2001; Sutton *et al*. 2006). The reported colour variation of *L. atritus* occurs within the overlap (McCutcheon 1976), therefore abdominal colouration is not a consistent difference in the morphology between *L. katipo* and *L. atritus*. We suggest that abdominal colouration is clinal over latitude within one species. A similar trend of abdominal colour pattern variation can be seen in the corinnid *Castianeira descripta* (Hentz, 1847) in North America (Reiskind 1969). Specimens of *C. descripta* in the southern areas of its distribution have distinct red markings that almost cover the entire abdomen and specimens at the northern limit of its distribution are almost entirely black (Reiskind 1969). Darker morphs of *C. descripta* are found in cooler climates, where heat absorption would be advantageous. New Zealand *Latrodectus*

Table 4. Leg 1 tibia-patella and carapace measurements for 21 female *Latrodectus atritus* specimens
AMNZ, Auckland Museum, New Zealand; MONZ, Museum of New Zealand Te Papa Tongarewa, New Zealand

Locality details	Tibia–patella 1 length	Carapace length	Ratio tibia–patella/ carapace length
NZ, BP, Matakana Is (37°31′S, 176°02′E), 17.xi.2004, M.E. Sutton. MONZ.	4.41	2.90	1.52
NZ, CL, Kaitoke Beach, Great Barrier Is (36°15′S, 175°29′E), 4.xi.2001, J.W. Early, R.F. Gilbert. AMNZ 6715.	4.48	2.80	1.60
NZ, CL, Kaitoke Beach, Great Barrier Is (36°15′S, 175°29′E), 4.xi.2001, J.W. Early, R.F. Gilbert. AMNZ 6715.	4.83	2.93	1.65
NZ, CL, Kaitoke Beach, Great Barrier Is (36°15′S, 175°29′E), 4.xi.2001, J.W. Early, R.F. Gilbert. AMNZ 6715.	4.38	2.65	1.65
NZ, CL, Okiwi Estuary, Great Barrier Is (36°9′S, 175°23′E), 6.xi.2001, R.F. Gilbert, J.W. Early. AMNZ 6716.	4.34	2.63	1.65
NZ, CL, Waihi Beach (37°24′S, 175°57′E), 13.vi.2004, JWG. MONZ. (Molecular specimen *La*2)	4.41	2.99	1.47
NZ, CL, Waihi Beach (37°24′S, 175°57′E), 13.vi.2004, JWG. MONZ. (Molecular specimen *La*3)	4.41	2.83	1.56
NZ, CL, Whangapoua Bush, Great Barrier I (36°8′S, 175°25′E), 20.ii.2002, J.W. Early. AMNZ 64304.	4.11	2.52	1.63
NZ, CL, Whangapoua Bush, Great Barrier I (36°8′S, 175°25′E), 20.ii.2002, J.W. Early. AMNZ 64304.	4.00	2.49	1.61
NZ, CL, Whangapoua Bush, Great Barrier I (36°8′S, 175°25′E), 20.ii.2002, J.W. Early. AMNZ 64304.	4.68	2.94	1.59
NZ, CL, Whangapoua Bush, Great Barrier I (36°8′S, 175°25′E), 20.ii.2002, J.W. Early. AMNZ 64304.	4.02	2.68	1.50
NZ, CL, Whangapoua Bush, Great Barrier I (36°8′S, 175°25′E), 20.ii.2002, J.W. Early. AMNZ 64304.	4.38	2.89	1.52
NZ, GB, Pouawa Reserve (38°37′S, 178°11′E), 4.vii.2004, JWG. MONZ. (Molecular specimen *La*1)	4.37	2.68	1.63
NZ, ND, Hukatere, Ninety Mile Beach (34°54′S, 173°5′E), 22.vii.1975, C.L. Wilton. MONZ.	4.29	2.76	1.55
NZ, ND, Spirits Bay, Kapowairua (34°26′S, 172°51′E), 18.xii.1996, J.W Early, R.B. Early. AMNZ 6106.	4.26	2.56	1.66
NZ, ND, Spirits Bay, Kapowairua (34°26′S, 172°51′E), 18.xii.1996, J.W. Early, R.B. Early. AMNZ 6105.	4.09	2.50	1.64
NZ, AK, Tawharanui Peninsula (36°23′S, 174°50′E), 3.i.1997, J.W. Early. AMNZ 6101.	4.43	2.89	1.53
NZ, AK, Tawharanui Peninsula (36°23′S, 174°50′E), 10.i.1986, D.J. Court. AMNZ 6442.	4.80	2.96	1.62
NZ, AK, Tawharanui Peninsula (36°23′S, 174°50′E), 18.xii.1996, J.W. Early, R.B. Early. AMNZ 6103.	3.86	2.54	1.52
NZ, TK, New Plymouth, Bell Block Dunes (39°2′S, 174°9′E), 2.iii.1979, E.R. McCutcheon. MONZ.	4.27	2.69	1.59
NZ, WO, Port Waikato (37°23′S, 174°44′E), 17.xi.1998, J.W. Early, R.F. Gilbert, T.L. Villard. AMNZ 6494.	4.04	2.62	1.54
Mean	4.33	2.74	1.58

Table 5. Morphological comparison between *Latrodectus atritus* ($n = 13$ (3 males, 10 females)) and *L. katipo* ($n = 12$ (3 males, 9 females))
Cara/Ster/Lab = carapace/sternum/labium; L/W = length/width; PME/PLE/AME/ALE = posterior median/posterior lateral/anterior median/anterior lateral eye diameters; PME-PME/PME-PLE/AME-AME/AME-ALE = distance between eyes; Fe/Pa/Ti/Mt/Ti = femur/patella/tibia/metatarsus/tarsus; # = leg number; P = pedipalp. Specimens listed in Table 6

Sex	Cara L	Cara W	PME-PME	PME-PLE	AME-AME	AME-ALE	AME	PME	ALE	PLE	Ster L	Ster W	Lab W
Females													
L. katipo mean	2.798	2.569	0.184	0.180	0.148	0.142	0.151	0.167	0.156	0.167	1.659	1.444	0.674
L. katipo SD	0.220	0.189	0.016	0.017	0.019	0.017	0.015	0.008	0.017	0.014	0.095	0.085	0.080
L. atritus mean	2.735	2.504	0.180	0.189	0.146	0.142	0.153	0.166	0.156	0.178	1.609	1.378	0.642
L. atritus SD	0.275	0.224	0.021	0.016	0.010	0.020	0.018	0.011	0.018	0.013	0.129	0.102	0.063
	Lab L	Fe1	Pa1	Ti1	Mt1	Ta1	Fe2	Pa2	Ti2	Mt2	Ta2	Fe3	Pa3
L. katipo mean	0.343	3.924	1.264	3.089	3.834	1.488	2.826	1.111	1.914	2.618	1.063	2.397	1.013
L. katipo SD	0.033	0.147	0.129	0.134	0.216	0.064	0.181	0.107	0.135	0.138	0.057	0.130	0.071
L. atritus mean	0.316	3.816	1.373	2.937	3.718	1.425	2.841	1.158	1.858	2.493	1.044	2.337	1.013
L. atritus SD	0.036	0.256	0.175	0.187	0.277	0.117	0.209	0.094	0.134	0.152	0.102	0.152	0.128
	Ti3	Mt3	Ta3	Fe4	Pa4	Ti4	Mt4	Ta4	FeP	PaP	TiP	TaP	
L. katipo mean	1.418	2.106	0.862	3.803	1.361	2.558	3.621	1.183	0.746	0.382	0.485	0.734	
L. katipo SD	0.093	0.103	0.072	0.190	0.067	0.279	0.149	0.251	0.044	0.031	0.026	0.080	
L. atritus mean	1.383	2.034	0.918	3.737	1.324	2.493	3.516	1.229	0.766	0.386	0.507	0.771	
L. atritus SD	0.123	0.131	0.088	0.225	0.107	0.244	0.362	0.161	0.068	0.026	0.055	0.057	
Males													
L. katipo mean	1.5696	1.3107	0.1260	0.1016	0.1138	0.0569	0.1098	0.1260	0.1098	0.1179	0.9223	0.8091	0.3236
L. katipo SD	0.2294	0.1284	0.0186	0.0070	0.0141	0.0070	0.0122	0.0141	0.0122	0.0070	0.0485	0.0742	0.0280
L. atritus mean	1.4913	1.3754	0.1179	0.0935	0.1057	0.0610	0.1057	0.1220	0.1057	0.1179	0.9223	0.8091	0.3074
L. atritus SD	1.5006	0.0280	0.0070	0.0070	0.0070	0.0000	0.0070	0.0000	0.0070	0.0070	0.0485	0.0280	0.0280
	Lab L	Fe1	Pa1	Ti1	Mt1	Ta1	Fe2	Pa2	Ti2	Mt2	Ta2	Fe3	Pa3
L. katipo mean	0.1375	2.7832	0.7929	2.2330	2.8317	1.1489	1.8770	0.6472	1.3997	1.7961	0.8252	1.4239	0.5178
L. katipo SD	0.0140	0.2674	0.0280	0.3501	0.3410	0.0742	0.2491	0.0742	0.1147	0.2116	0.0485	0.1011	0.0742
L. atritus mean	0.1456	2.8641	0.8091	2.4919	2.9935	1.1974	1.9417	0.6958	1.4887	1.8932	0.8252	1.5534	0.5825
L. atritus SD	0.0000	0.1456	0.0280	0.1560	0.1011	0.0280	0.0971	0.0280	0.0742	0.0841	0.0000	0.1456	0.0485
	Ti3	Mt3	Ta3	Fe4	Pa4	Ti4	Mt4	Ta4	FeP	PaP	TiP	TaP	
L. katipo mean	0.9385	1.2945	0.6230	2.6052	0.6796	1.8608	2.4919	0.9709	0.3317	0.2265	0.2427	0.3560	
L. katipo SD	0.1011	0.1705	0.0852	0.2674	0.0971	0.2760	0.2926	0.0971	0.0140	0.0140	0.0000	0.0280	
L. atritus mean	0.9871	1.3916	0.6796	2.6537	0.7443	1.9741	2.5405	1.0194	0.3398	0.2346	0.2427	0.3722	
L. atritus SD	0.0742	0.0742	0.0000	0.1011	0.0280	0.0280	0.1222	0.0485	0.0000	0.0140	0.0000	0.0280	

specimens are darker in warmer climates, which seems counterintuitive. However, there is a significant relationship between mean annual temperature and abdominal colouration. Although the exact nature of this relationship is unclear, it would seem unlikely that it is to facilitate heat absorption. The relationship between colour and temperature could be tested by raising the eggsacs and spiderlings from spiders with a range of different coloured abdomens under a variety of temperature schemes.

Aside from abdominal colour, which has been reported to vary (McCutcheon 1976), there is no consistent difference in the morphology between *L. katipo* and *L. atritus*. Although this overall trend is clear, a considerable amount of intraspecific morphological variation was observed, even among individuals from the same population. For example, the patella–tibial index ranged from 1.50 to 1.63 for specimens collected at Whangapoua Bush. The density of abdominal setae on females varied between individual specimens, even from the same population. The density of setae in most specimens of *L. katipo* and *L. atritus* was typically like that shown in Fig. 3*J*, but a lower density of setae (Fig. 3*E*) was not uncommon in both species. Griffiths *et al*. (2005) found no difference between the genitalic structures of *L. katipo* and *L. atritus* and our overall findings corroborate their observations. However, there was some occasional variation in the arrangement of the sclerites of the male pedipalps (see Fig. 3*B* and *G*) although these variations were not consistent between the species. Apparent differences in the arrangement of the embolus (Fig. 3*A*, *F*) could also be due to the embolus not returning to its pre-copulatory arrangement (Levi 1959). There were also occasional variations in the shape of the coiled connecting duct (Fig. 3*D*, *I*), but again, variation in these traits was not consistent with colour pattern.

There is no doubt that New Zealand *Latrodectus* species and *L. hasseltii* are different species. We found consistent differences in COI and ITS sequences (Tables 2 and 3) and in the type and arrangement of abdominal setae on females (Fig. 3). There are also differences in the male pedipalp, and the external and internal genitalia (Fig. 3), but further examination of *L. hasseltii* specimens from throughout Australia would be needed to confirm whether these differences are consistent. Previous studies have found that New Zealand *Latrodectus* species and *L. hasseltii* differ behaviourally (Forster 1992, 1995), morphologically (Kavale 1986; Forster and Forster

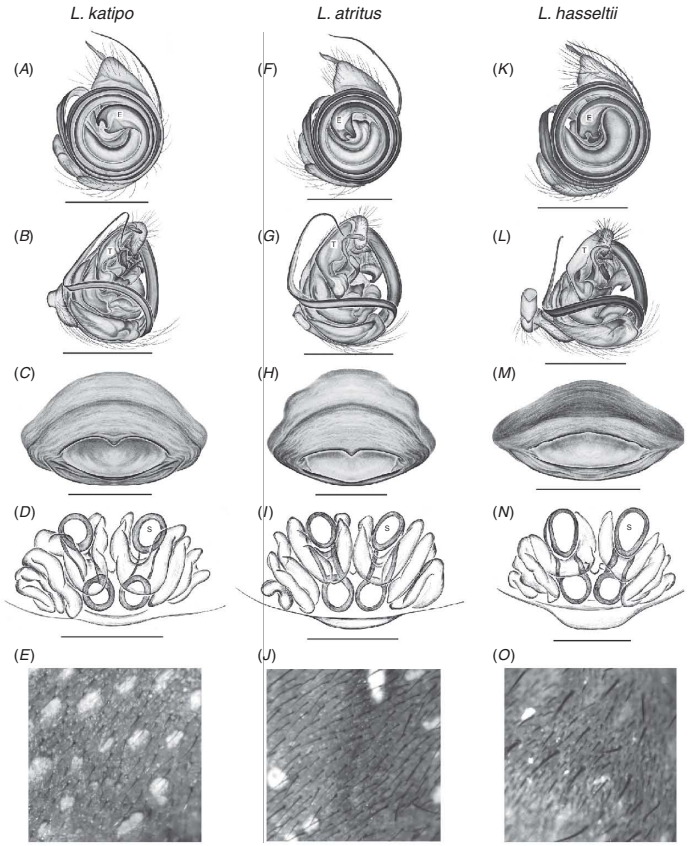

Fig. 3. *Latrodectus katipo* (*A–E*), *Latrodectus atritus* (*F–J*), *Latrodectus hasseltii* (*K–O*). (*A*, *F*, *K*) subectal view of left male pedipalps; (*B*, *G*, *L*) ventral view of left male pedipalps; (*C*, *H*, *M*) ventral view of epigynes; (*D*, *I*, *N*) dorsal view of epigynes; (*E*, *J*, *O*) abdominal setae, 1 mm square. (*A*, *B*) *Latrodectus katipo* (Motueka Spit (41°06′S, 173°02′E), Entomology Research Museum, Lincoln University, New Zealand (LUNZ)). (*C*, *D*) *Latrodectus katipo* (Waitarere Beach (40°33′S, 175°12′E), Museum of New Zealand Te Papa Tongarewa, New Zealand (MONZ)). (*E*) *Latrodectus katipo* (Kaitorete Spit (43°49′S, 172°40′E), LUNZ). (*F*, *G*) *Latrodectus atritus* (Tapotupotu Bay (34°26′S, 172°43′E), Auckland Museum, New Zealand (AMNZ) 6110). (*H*, *I*) *Latrodectus atritus* (Kaitoke Beach, Great Barrier I (36°15′S, 175°29′E), AMNZ 6715). *J*, *Latrodectus atritus* (Papamoa Beach (37°43′S, 176°16′E), LUNZ). (*K*, *L*) *Latrodectus hasseltii* (Alexandra (45°15′S, 169°24′E), Biosecurity Group, AgResearch, New Zealand (AgR) 9616). (*M*, *N*) *Latrodectus hasseltii* (Letts Gully Road, near Alexandra (45°14′S, 169°24′E), MONZ). (*O*) *Latrodectus hasseltii* (Mitchell Park, Adelaide (35°01′S, 138°34′E), AgR 9385). Scale bars 0.5 mm. Abbreviations used: E, base of embolus; T, theridioid tegular apophysis; S, spermatheca. (*A–J*) exemplify some of the variation seen in the male pedipalps, female genitalia and abdominal setae density and these can be seen in either *Latrodectus katipo* or *Latrodectus atritus*.

1999) and phylogenetically (Garb *et al*. 2004; Griffiths *et al*. 2005). Even so, the COI divergence is very low (3.3–4.3%), which is lower than the divergence in 97.6% of 1249 closely related chelicerate species surveyed by Hebert *et al*. (2003). The maximum COI divergence found between *L. katipo* and *L. atritus* was 1.2% and the mean was under 1%.

Table 6. Material examined for general morphology measurements
AMNZ, Auckland Museum, New Zealand; MONZ, Museum of New Zealand Te Papa Tongarewa, New Zealand; f, female; m, male

Species	Sex	Locality details
Latrodectus atritus	f	NZ, ND, Spirits Bay, Kapowairua (34°26′S, 172°51′E), 18.xii.1996, J.W. Early, R.B. Early. AMNZ 6104.
	f	NZ, ND, Spirits Bay, Kapowairua (34°26′S, 172°51′E), 18.xii.1996, J.W. Early, R.B. Early. AMNZ 6105.
	m	NZ, ND, Tapotupotu Bay (34°26′S, 172°43′E), 19.xii.1996, J.W. Early. AMNZ 6110.
	f	NZ, AK, Tawharanui Peninsula (36°23′S, 174°50′E), 18.xii.1996, J.W. Early, R.B. Early. AMNZ 6103.
	f	NZ, AK, Tawharanui Peninsula (36°23′S, 174°50′E), 3.i.1997, J.W. Early. AMNZ 6101.
	2f, m	NZ, CL, Whangapoua Bush, Great Barrier I (36°8′S, 175°25′E), 20.ii.2002, J.W. Early. AMNZ 6892.
	f	NZ, CL, Okiwi Estuary, Great Barrier I (36°10′S, 175°23′E), 6.xi.2001, R.F. Gilbert, J.W. Early. AMNZ 6716.
	f	NZ, WO, Port Waikato (37°23′S, 174°44′E), 17.xi.1998, J.W. Early, R.F. Gilbert, T.L. Villard. AMNZ 6494.
	f, m	NZ, BP, Matakana Is (37°31′S, 176°02′E), 17.xi.2004, M.E. Sutton. MONZ.
	f	NZ, TK, New Plymouth, Bell Block dunes[A] (39°2′S, 174°9′E), 2.iii.1979, E.R. McCutcheon. MONZ.
Latrodectus katipo	f	NZ, TK, New Plymouth, Bell Block dunes[A] (39°2′S, 174°9′E), 3.iii.1988, E.R. McCutcheon. MONZ.
	f	NZ, TK, New Plymouth, Bell Block dunes[A] (39°2′S, 174°9′E), 3.iii.1985, E.R. McCutcheon. MONZ.
	f	NZ, WI, Wanganui (39°56′S, 175°2′E), viii.1986, M. Ordish. MONZ.
	f, m	NZ, WN, Levin, Waitarere Beach (40°33′S, 175°12′E), 17.iv.1954, R.G. Ordish. MONZ.
	f	NZ, WN, Otaki Beach (40°45′S, 175°7′E), 27.xii.1985, R.K. Dell. MONZ.
	f	NZ, WA, Mataikona (40°47′S, 176°16′E), 20.i.1974, Allpress. MONZ.
	f	NZ, WN, Paraparaumu (40°53′S, 174°58′E), 15.v.1949. MONZ.
	f	NZ, WN, Miramar, Wellington (41°19′S, 174°49′E), 20.ix.1939. Mr Johnson. MONZ.
	2m	NZ, MC, Kaitorete Spit (43°49′S, 172°40′E), 10.iii.2005, A.M. Evans. MONZ. (Includes molecular specimen *Lk*11).
	f	NZ, SC, near Orari River mouth (44°14′S, 171°25′E), 6.ix.2007, CJV, W.G. Chinn, D. Anderson. MONZ. (Molecular specimen *Lk*19).

[A]Both *L. atritus* and *L. katipo* are found at Bell Block dunes.

Hebert *et al*. (2003) found no closely related chelicerate species with a COI divergence less than 1%. What was surprising was that COI divergence among the *L. hasseltii* specimens we sampled was lower than 0.5%, despite collection localities being over 4000 km apart. These low levels of COI divergence support the hypothesis that *L. hasseltii* might not be endemic to Australia (Raven and Gallon 1987). Raven and Gallon (1987) questioned the endemic status of *L. hasseltii* because redbacks are most common in urban areas and had not been recorded in Australia before 1870, when about 200 spider species had already been found. However, Main (1993) reported that *L. hasseltii* were known from South Australia in 1850 and Downes (1993) suggested that redbacks were known to the aboriginal people of Australia. More sampling, including from South Australia, may well uncover greater genetic variation in *L. hasseltii*.

It could be argued that incomplete lineage sorting has occurred in New Zealand *Latrodectus* species and it is often impossible to demonstrate conclusively that incomplete sorting explains any particular case of polyphyly (Funk and Omland 2003). However, we used two loci (COI, ITS), which increases the probability of detecting reciprocal monophyly (Knowles and Carstens 2007). In addition, we examined morphology and conducted limited cross-breeding experiments to further increase the possibility of detecting consistent differences between *L. katipo* and *L. atritus*. Therefore, we believe that our data show that there is one endemic species of *Latrodectus*, *L. katipo*, in New Zealand with genetic and ecological exchangeability owing to the species' mobility and that the colour differences are a latitudinal, clinal variation, correlated to mean annual temperature.

Taxonomy

Family **THERIDIIDAE** Sundevall

Genus ***Latrodectus*** Walckenaer

Latrodectus katipo Powell

(Fig. 3*A*–*J*)

Latrodectus katipo Powell, 1871: 57, pl. 5, figs a–g. 80. – Urquhart, 1892: 224; Pickard-Cambridge, 1902*a*: 39; Pickard-Cambridge, 1902*b*: 255, 258, pl. 27, fig. 5; Dahl, 1902: 42; Dalmas, 1917: 360; Bryant, 1933: 11; Gerschman & Schiapelli, 1942: 5, 10, 22, fig. 2 (5a–5e), as *L. tredecimguttatus katipo* and *L. katipo*, removed from synonymy of *L. hasseltii*; Parrott, 1946: 70; Keegan, 1955: 149, fig. 17; Forster & Forster, 1970: 153, unnumbered figs; Forster & Forster, 1973: 231, figs 157–159; Forster, 1975: 502; McCutcheon, 1976: 204; Forster & Kingsford, 1983: 432; McCutcheon, 1992: 1; Forster, 1995: 22; Forster & Forster, 1999: 173, figs 12.5–12.8, not fig. 12.5b; Crowe, 2007: 20, unnumbered figs.

Latrodectus scelio Thorell, 1870, in part: 370. – Thorell, 1881: 178; Urquhart, 1894: 218.

Theridium melanozantha Urquhart, 1887: 102, pl. 8, fig. 8.

Theridium zebrinia Urquhart, 1890: 256.

Latrodectus katipo var. *atritus* Urquhart, 1890: 259, **syn. nov.** – Urquhart, 1892: 224, as *L. katipo* var. *attritus* [sic]; Hutton, 1904: 239, as *L. hasselti* [sic] var. *atritus*; Dalmas, 1917: 360, elevated to subspecies; Parrott, 1948: 162, as *L. hasseltii atritus*; Forster, 1975: 502, elevated to species; McCutcheon, 1976: 204; Forster & Kingsford, 1983: 432; McCutcheon, 1992: 1; Forster, 1995: 23; Forster & Forster, 1999: 173, fig. 12.5b; Crowe, 2007: 20, unnumbered figs.

Latrodectus hasseltii Thorell, 1870, in part: 369. – Hutton, 1904: 239, as *L. hasseltii* [sic]; Roewer, 1942: 426; Parrott, 1948: 162, as *L. hasseltii hasseltii*; Bonnet, 1957: 2369, as *L. hasseltii* [sic].

Latrodectus mactans (Fabricius, 1775), in part. – Levi, 1959: 24.

Steatoda sp. "black katipo" Forster & Forster, 1973: 229.

Type material

Type(s) of Latrodectus katipo. ♀, New Zealand (considered lost, not examined).

Syntypes of Theridium melanozantha. 6 ♂, Waiwera, New Zealand (36°33′S, 174°43′E), A.T. Urquhart (CMNZ, examined).

Holotype of Theridium zebrinia. 1 immature ♀, Wellington, New Zealand (41°17′S, 174°47′E), T. Kirk (CMNZ, examined).

Holotype of L. katipo var. atritus. ♀, Portland Island, New Zealand (39°17′S, 177°52′E) (CMNZ, examined).

Other material examined

New Zealand: ND: 2♀ Spirits Bay (AMNZ 6105, 6106); 1♀ Rarawa Bay (MONZ); 1♀ Hukatere (MONZ); 1♂ Tapotupotu Bay (AMNZ 6110). **AK:** 3♀ Tawharanui Peninsula (AMNZ 6101, 6103, 6442); 1♀, 1 immature ♀ Pakiri Beach (MONZ). **WO:** 1♀ Port Waikato (AMNZ 6494). **CL:** 3♀ Kaitoke Beach (AMNZ 6715); 1♀ Okiwi Estuary (AMNZ 6716); 1♂, 7♀ Whangapoua Bush (AMNZ 64304, 6892); 1♀ Opoutere Beach (MONZ); 2♀ Waihi Beach (MONZ). **BP:** 2♂, 1♀ Matakana Island (MONZ); 1 immature Papamoa Beach (MONZ); 2♂, 2♀ Papamoa Beach (LUNZ). **GB:** 1♀ Pouawa Reserve (MONZ); 1♀ Houpoto (MONZ). **TK:** 3♀ Bell Block dunes (MONZ). **WI:** 1♀ Waiinu Beach (MONZ); 1♀ Wanganui (MONZ); 1 immature ♀ Whitiau Scenic Reserve (MONZ); 1♀ Himatangi Beach (MONZ). **WN:** 1♂, 1♀ Waitarere Beach (MONZ); 1♀ Otaki Beach (MONZ); 1♀ Paraparaumu (MONZ); 1♀ Miramar (MONZ). **WA:** 1♀ Mataikona (MONZ); 2♀ Herbertville (MONZ); 3♀ Flat Point (MONZ). **NN:** 2♀ Farewell Spit (MONZ); 1♂, 1♀ Motueka Spit (LUNZ). **NC:** 2♀ Waikuku Beach (LUNZ). **MC:** 2♂, 1♀ Kaitorete Spit (MONZ); 3♂, 2♀ Kaitorete Spit (LUNZ). **SC:** 1♂, 1♀ near Orari River mouth (MONZ).

Diagnosis

Latrodectus katipo differs from its sister-species, *L. hasseltii*, by the following characters: the abdomen of the female is covered in short fine setae (*L. hasseltii* has both long fine setae and stouter short setae); the female patella–tibial index (the total length of the patella and tibia of leg I divided by the carapace length from the clypeal apex to the posterior notch) is 1.58 (it is 1.86 in *L. hasseltii*); in the male pedipalp, the edge of the theridioid tegular apophysis (TTA) is curled ventrally so that the knob-like projections on the dorsal surface of the TTA are visible at the anterior and ectal edges of the TTA when viewed ventrally (the knob-like projections are not visible in the ventral view of *L. hasseltii*); a triangular-shaped sclerotised area at the base of the embolus (can be present or absent in *L. hasseltii*); in the female genitalia, the rounded anterior end of the spermatheca is approximately one-third of the total length of the spermatheca (approximately one-half the total length of the spermatheca in *L. hasseltii*); there is a posteriorly directed hump at the centre of the anterior edge of the depression of the external genitalia (the posteriorly directed hump can be present, reduced or absent in *L. hasseltii*).

DNA sequences

Mitochondrial ND1 (GenBank accession numbers AY383604–AY383611) and COI (AY383052, AY383053) DNA sequences were reported in Griffiths *et al*. (2005) and Garb *et al*. (2004) respectively. Here we report new mitochondrial COI (EF121007–EF121030, EU305448–EU305455, EU309678) and nuclear ITS1 and ITS2 (EF121038–EF121061, EU305456–EU305463, EU309679) DNA sequences. The mitochondrial COI DNA sequence (EF121006) from Pouawa Reserve is not typical of *L. katipo* and is likely to be the result of introgression with *L. hasseltii*.

Remarks

The taxonomy of *Latrodectus* has suffered from much confusion (see Levi 1983) and the taxonomy of *L. katipo* is no exception. Ten years after the initial description of *L. katipo*, Thorell (1881) placed it in synonymy with his own *L. scelio* (=*L. hasseltii*), a conclusion with which Urquhart (1894) agreed. In the meantime, Urquhart (1890) published a brief description of a black form of katipo as *Latrodectus katipo* var. *atritus*. Pickard-Cambridge (1902*b*: 255) considered *L. katipo* to be a subspecies, but he did not specify the species in which it was included (p. 251). Hutton (1904) listed *L. hasseltii* for New Zealand rather than *L. scelio*, presumably following Pickard-Cambridge (1902*b*). However, Hutton (1904) did not follow Pickard-Cambridge's treatment of *L. katipo* and *L. hasseltii* as separate taxa, and retained *L. atritus* as a variety of *L. hasseltii*. Taxonomic works by Dahl (1902), Dalmas (1917) and Bryant (1933) treated *L. katipo* as a distinct species. Dalmas (1917) also elevated *L. k.* var. *atritus* to a subspecies of *L. katipo*, whereas Bryant (1933) synonymised two species, *Theridium melanozantha* and *T. zebrinia* (as *T. zelrina* [sic]) under *L. katipo*. Roewer (1942) listed *L. katipo* as a synonym of *L. hasseltii*, but did not include *L. atritus*. Parrott (1946) observed the differences of opinion over the status of whether or not *L. katipo* was distinct from *L. hasseltii*, but he retained the latter as a separate species, recognising that both species needed to be compared to resolve the matter. Subsequently, Parrott (1948) did compare the two taxa, synonymising *L. katipo* under *L. hasseltii*, and regarding the black katipo as a subspecies of the latter. Keegan (1955) erroneously stated that Pickard-Cambridge (1902*b*) considered *L. katipo* to be a subspecies of *L. tredecimguttatus* Rossi, 1790 when, in fact, Pickard-Cambridge (1902*a*: 251) declined to place any of his so-called subspecies under any species, *L. katipo* included. Keegan may have been confused by the arrangement of Pickard-Cambridge's (1902*b*: 251) table, which is more accurately described as a key, listing *L. katipo* below *L. tredecimguttatus*. However, given Pickard-Cambridge's earlier statement on his reluctance to place subspecies, this cannot be interpreted as a taxonomic arrangement. Indeed, later that year, Pickard-Cambridge (1902*a*) published another key with a completely different arrangement as a supplement to his earlier revision. Keegan's (1955) misinterpretation of Pickard-Cambridge (1902*b*) also seems to have missed the latter's inconsistent position on the status of *L. tredecimguttatus*. On one hand, Pickard-Cambridge (1902*b*) suggested that *L. tredecimguttatus* is probably a good species (p. 251) yet later on he categorised it as a subspecies (p. 254). Bonnet (1957) listed both *L. atritus* and *L. katipo* as synonyms of *L. hasseltii*.

Levi (1959), in his worldwide revision of *Latrodectus*, reduced the total number of species to six and considered both *L. katipo* and *L. hasseltii* to be synonyms of *L. mactans*. Subsequent authors based in New Zealand (e.g. Forster and Forster 1973; Forster 1975; McCutcheon 1976; Forster and Kingsford 1983) rejected Levi's (1959) treatment of *L. katipo*, resurrecting it as a full species, which was vindicated when Levi (1983) acknowledged that his earlier treatment of *Latrodectus* was incorrect. Levi (1983) did not, however, formally reinstate *L. katipo* or other species he had synonymised. The species name, *L. katipo*, has been in common usage for the katipo spider in the New Zealand literature since 1970. Although Forster and Forster (1973) thought that the black katipo might be a species of *Steatoda* Sundevall, 1833, in subsequent papers written both jointly and singly, they used *L. atritus* for this form (e.g. Forster 1975, 1995; Forster and Kingsford 1983; Forster and Forster 1999).

Note that despite the frequent usage of the name *Latrodectus hasselti*, the correct original spelling is *Latrodectus hasseltii* Thorell, 1870. This is in accordance with article 33.4 in the fourth edition of the *International Code of Zoological Nomenclature* (International Commission on Zoological Nomenclature 1999), which states the change from *-i* to *-ii* is a subsequent incorrect spelling. There is provision in the *International Code of Zoological Nomenclature* for prevailing usage of an unjustified emendation (article 33.2.3.1) or incorrect subsequent spelling (article 33.3.1); however, *hasselti* is not an emendation but an incorrect subsequent spelling and article 33.4 is an exception to articles 33.2.3.1 and 33.3.1. Using Google searches (www.google.co.nz; verified November 2008), we found that both spellings are in frequent use and, as article 33.4 is quite clear on the matter, we advocate use of the correct original spelling. We have included both spellings in the keywords of this paper to facilitate searches.

Conservation implications

A reliable taxonomic basis is fundamental for adequate management (Paquin *et al*. 2008). Our study shows that there is one endemic New Zealand *Latrodectus* species, *L. katipo*, which has intraspecific colour variation correlated with an environmental variable (annual mean temperature at location).

The presence of *L. hasseltii* COI sequence in a specimen of *L. katipo* (*La*1 from Pouawa Reserve – see Fig. 1) coupled with that specimen's *L. katipo* ITS sequence and morphology suggests introgression between the two species. Mitochondrial DNA is maternally inherited; therefore, a female in specimen *La*1's matrilineal lineage will have had to have been a *L. hasseltii* that had successfully mated with either a male *L. katipo* or a male hybrid of *L. katipo* and *L. hasseltii*. Laboratory studies have shown that *L. hasseltii* females will not mate with *L. katipo* males (Kavale 1986; Forster 1992, 1995), in which case a mating between *L. hasseltii* and a hybrid would be the most likely scenario. This would require a male and a female *L. hasseltii* to have been introduced to the Pouawa Reserve population. We cannot be sure as to how many generations ago this mating occurred. However, we did not observe any heterozygous nucleotide sites in the ITS sequence, which would suggest the interspecific mating occurred at least two generations ago, assuming a simple Mendelian inheritance pattern and that there was no subsequent interspecific mating. Further sampling at Pouawa Reserve would be required to ascertain the extent of introgression in the population there. This introgression between the two species has serious conservation implications for *L. katipo*. *Latrodectus hasseltii* is frequently intercepted among imports at New Zealand ports and has established in Central Otago and near New Plymouth (Forster 1984; McCutcheon 1992; Forster and Forster 1999). Efforts should be made to prevent further establishment of *L. hasseltii* at sites near *L. katipo* populations as introgression may homogenise the two species over time. Ideally all populations of *L. hasseltii* should be eliminated in New Zealand, although this may not be possible in Central Otago, where it appears to be widespread (Forster 1984; Forster and Forster 1999; C. J. Vink, unpubl. data).

We recommend that the Department of Conservation consider all endemic New Zealand *Latrodectus* species as one species, *L. katipo*, and that efforts be made to prevent further introgression with *L. hasseltii*. *Latrodectus katipo* should retain its New Zealand Department of Conservation classification as in serious decline.

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Appendix F

Published research II

The following research study and the published paper were the result of a collaboration in Kathrin Affeld's project during the Ph.D..

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The invertebrate fauna of epiphyte mats in the canopy of northern rata (Myrtaceae: *Metrosideros robusta* A. Cunn.) on the West Coast of the South Island, New Zealand

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Abstract The range of microhabitats and microclimatic conditions provided by epiphytes has been linked to the high diversity of invertebrates in many forest canopies worldwide, but comparably little is known about the invertebrate fauna in this habitat in New Zealand. This study compiled an inventory of the invertebrate fauna of epiphyte mats in the canopy of northern rata (Myrtaceae: *Metrosideros robusta* A. Cunn.) at two study sites on the West Coast of the South Island. A total of 242 069 invertebrate specimens was collected over one year, representing 4 phyla, 9 classes and more than 160 families, 225 genera and 446 species. At least 10 new species and 3 new genera were identified, while 5 species were recorded outside their known geographical range. Epiphyte mats provided habitat for an invertebrate fauna, highly diverse and abundant both taxonomically and functionally, dominated in terms of abundance by Acari, Collembola and Hymenoptera (largely ants), and in terms of feeding guilds by epiphyte grazers and ants. As the first inventory of this taxonomic depth and breadth compiled for New Zealand forest epiphyte habitats, this study provides important baseline data for the conservation of biodiversity in New Zealand's indigenous forests.

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INTRODUCTION

Invertebrates represent an estimated 2–6 million (Novotny et al. 2002) of all species on Earth, and of these c. 20–25% (Sørensen 2003) are found in the canopies of tropical and temperate rainforests. This exceptionally high diversity has been linked to epiphytes and the wide range of microhabitats and microclimatic conditions provided by forest canopies (Benzing 1990; Kitching et al. 1997).

New Zealand has one of the most diverse and extensive epiphyte flora of any temperate forest system (Benzing 1995; Dawson & Lucas 2005), but little is known about the diversity of the invertebrate fauna associated with these epiphytic canopy habitats. Such information, however, is crucial for understanding the spatial and seasonal distribution of New Zealand's highly endemic invertebrate fauna and its involvement in the functioning of indigenous forest ecosystems.

Most of the few existing canopy studies in New Zealand have focused on airborne arboreal arthropods, particularly Diptera (Moeed & Meads 1984; Didham 1992, 1997; McWilliam & Death 1998; Ewers et al. 2002). These studies have provided valuable information on the influence of canopy habitat characteristics, season and the effects of fragmentation on the structure and distribution of the communities, but little on the origin or residence of the recorded species and their actual distribution and habitat use in the canopy. Indeed, a study by Derraik & Heath (2005) indicated discrete assemblages of immature Diptera in different types of phytotelmata habitats in the North Island of New Zealand. Distinct differences in the species composition and abundance of invertebrate communities have also been shown for structurally different epiphyte habitats, such as epiphyte mats and phytotelmata in rimu (*Dacrydium cuppressinum*) trees on the West Coast of the South Island (Affeld 2002), and orchid and tank bromeliad species in a tropical rain forest in Panama (Stuntz et al. 2002).

Clearly, compiling a complete species inventory of invertebrates in canopy habitats is a challenging task, particularly in New Zealand where only 6% of the estimated native non-arthropod invertebrate and 50–60% of the arthropod fauna has been described (Emberson 1998; Halloy et al. 2001). Nevertheless, invertebrate inventories from a range of canopy habitats and sites can provide important base-line data on the distribution of New Zealand's biodiversity, and thereby help to prioritise conservation areas.

The aim of this paper is to provide a detailed inventory of the canopy invertebrate fauna of epiphyte mats in northern rata (Myrtaceae: *Metrosideros robusta* A. Cunn.) on the West Coast of the South Island, an area that is generally poorly represented in invertebrate studies of any kind. This paper is also part of a larger study that investigated relationships between the composition and characteristics of epiphyte communities with their inhabitant invertebrate fauna (Affeld 2008; Affeld et al. 2008).

METHODS

Study site

This study was conducted at two coastal sites on the West Coast of the South Island of New Zealand: at Bullock Creek (42°06′S; 171°20′E), Punakaiki in the Paparoa National Park and at the Heaphy Track (41°10′S; 172°10′E), near Karamea in the Kahurangi National Park. The climate is mild and very humid throughout the year at both sites. Average daily minimum and maximum temperatures range from 7.6–13.8°C in winter (June–August) and 13.6–20.6°C in summer (December–February) in Punakaiki and 5.1–13.8°C in winter and 11.8–21°C in summer in Karamea (NIWA 2007). The average annual rainfall at Punakaiki is approximately 2600 and 1900 mm in Karamea (NIWA 2007). Both sites are covered in unlogged lowland rain forest vegetation consisting primarily of podocarp and broadleaf species that support a profusion of epiphytes and lianas. Northern rata is a common emergent canopy tree at both sites, and an important host for diverse epiphyte communities. In another part of this study that investigated the composition of epiphyte mats, 157 epiphyte species, comprising 32 vascular and 125 non-vascular species were identified (Affeld et al. 2008). *Hymenophyllum nephrophyllum*, *Astelia* sp., *Metrosideros perforata* and *Earina autumnalis* were among the most abundant vascular species, whereas the mosses *Hypnum chrysogaster* and *Macromitrium gracile* and the liverworts *Lepidolaena taylorii*, *Bazzania hochstetteri*, *Plagiochilion conjugatum* and *Porella elegantula* were the most abundant non-vascular species on northern rata.

Sample collection and processing

To compile detailed invertebrate inventories of epiphyte mats in canopy habitats, a total of 120 epiphyte mat samples was collected from 20 northern rata (*Metrosideros robusta*: Myrtaceae) trees at each of the two study sites. Sampling was conducted 3-monthly between April 2004 and April 2005. On each sampling occasion, 12 epiphyte samples consisting of 30 × 25 cm quadrats were collected from five of the 20 pre-selected trees at each site. In April 2004 and 2005 the same trees were sampled. Samples were located on the inner branches about 1.0–1.5 m from the main trunk at an average 20 m above the ground. On any given tree, either two or three samples were selected from a random subset of suitable and accessible epiphyte mats, but only one epiphyte mat was sampled per branch. Single rope techniques (Winchester 2004) were used to gain access to the canopy, while safety slings allowed for free movement between branches.

To study the invertebrates inhabiting epiphyte mats it was essential to sample the epiphyte habitats directly. Conventional methods such as insecticide fogging were considered unsuitable, since animals remaining inside funnel-shaped plants (Ellwood et al. 2002) or in the thick humus layer associated with many epiphytes (Yanoviak et al. 2003) are not captured. Each epiphyte sample was carefully detached from the bark and enclosed in a separate plastic bag to be transported to the lab for processing.

In the laboratory, invertebrates were extracted from the epiphyte material using Berlese funnels. Samples were kept in the funnels for a minimum of 3 days or until the sample material was completely dry. Subsequently, all organic matter was thoroughly washed over three stacked sieves of decreasing mesh size (1.7 mm, 500 µm and 75 µm mesh size) to extract any remaining invertebrates that were trapped among the dried epiphyte material. All epiphyte material was oven-dried for 72 h at 65ºC and the dry weight recorded.

All invertebrates were sorted to morphospecies within higher taxa, counted and preserved in 75% ethanol. Specimens from most orders were sent to expert taxonomists for further identification to the lowest possible taxonomic level. Morphospecies from the few (mainly minor) orders for which no taxonomic expertise was available were distinguished based on morphological characteristics used in various taxonomic keys. The majority of species could be assigned to family level, but only 16.2% could be identified at species or morphospecies level. Most of the identified species were insects, c. 22% of the insect fauna, and belonged to the Coleoptera, Lepidoptera, Hemiptera and Hymenoptera. In contrast, identification beyond family level was not possible for Diptera, Collembola and most non-insect taxonomic groups in this study. Overall, only 6.3% of all non-insect specimens could be identified to species level. A reference collection was deposited in the Entomology Museum at Lincoln University, Canterbury, New Zealand.

To gain an understanding of the function of the invertebrates in the canopy ecosystem, all specimens included in the species list were further quantified according to feeding guilds based on the guilds recognised by Moran & Southwood (1982) and Stork (1987). The guilds used in this study comprised herbivores, which were further split into chewers and sap feeders, scavengers (including dead wood, lichen and fungal feeders) and epiphyte grazers; predators; parasitoids; ants; and others. All epiphyte grazers in this study were Collembola, and although this group is involved in the breakdown of organic matter and the cycling of nutrients, Collembola were here classified as epiphyte grazers rather than scavengers, because most species feed on micro-organisms associated with the rhizosphere and decomposing organic matter rather than decayed plant material (Greenslade 1991). Species for which no information was available about their feeding habit, or species that could be assigned to more than one guild (except ants), were classified as "others".

Data analysis

To assess the completeness of the inventories, a sample-based rarefaction curve (with 100 random draws) was generated using EstimateS version 7.5 (Colwell 2005). Two non-parametric incidence-based species richness estimators, Chao2 and ICE (Colwell 2005) were used to account for potential bias from insufficient sampling (Magurran 2004). Both estimators use presence/absence data and give minimum estimates of species richness that are based on the number of rare species found in one and two samples only (Chao2) or in 10 or fewer sampling units (ICE) (Chazdon et al. 1998).

Two-sample *t*-tests were used to determine whether differences in invertebrate abundance or species richness were significant between sites or seasons. Acari are included in the inventory, but are omitted from the analysis because recording detailed distributional data for this group was beyond the scope of this study.

Simple linear regression was used to determine whether relationships existed between the abundance or presence/absence of the ant *Prolasius advena* and hemipteran coccids that are known to be tended by this ant species.

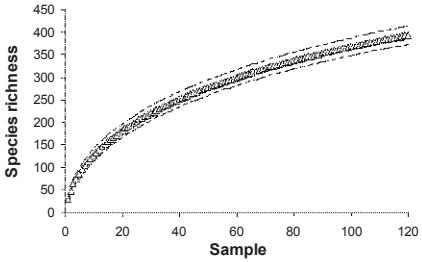

Fig. 1 Sample-based rarefaction curve and 95% confidence intervals for species richness of invertebrates (excl. Acari) collected from 120 epiphyte mats in the canopy of northern rata at two study sites.

RESULTS

Altogether, 242 069 individuals, representing 4 phyla (Annelida, Arthropoda, Mollusca and Nematoda), 9 classes (Arachnida, Chilopoda, Diplopoda, Entognatha, Gastropoda, Insecta, Malacostraca, Oligochaeta and Symphyla), more than 18 orders, 160 families and 446 invertebrate species/morphospecies (Appendix) (hereafter referred to as species) were collected from 120 epiphyte mats with a total epiphyte biomass of 30.6 kg across the two study sites. The sample-based rarefaction curve (Fig. 1) showed no sign of saturation, which indicates that not all species present were sampled, despite the high sampling effort. Incident-based species richness estimators ICE and Chao 2 (Colwell 2005) confirmed that the species inventory presented here is incomplete, and suggested that the expected species richness (excluding Acari) in these epiphyte mat habitats lies between 546 and 566 species. All higher taxonomic groups (to order level) were present at both sites except for Nematoda, which was not recorded at Karamea, and Dermaptera, which was not collected at Punakaiki. Species richness at the two sites was remarkably similar (316 species recorded at Punakaiki and 307 at Karamea), but only 198 species were found at both sites; 107 species were recorded in only one of all 120 collected samples. There was some seasonal fluctuation in species richness, ranging from 226 species in April 2004 to 183 species in January 2005 (Fig. 2A); 71 species (16.7%) were collected in all five sampling periods, while 192 species (45.2%) were represented only in autumn (April 2004 and April 2005), winter (July 2004), spring (October 2004) or summer (January 2005).

More specimens were collected at Punakaiki (129 782) than Karamea (112 287), but this difference was not significant ($t = 0.86$; $P > 0.05$). Acari dominated the invertebrate communities, comprising 58.1% of the total number of specimens collected, followed by Collembola (19.9%) and Hymenoptera (13.9%, mostly ants). The highest numbers were collected in April (24.2%), and the lowest number in July (14.4%) (Fig. 2B). Seasonal differences were particularly pronounced in Punakaiki, where invertebrate abundance was >50% lower in July compared with any other sampling period of the year.

Immature specimens contributed a small proportion (c. 5%) to overall invertebrate abundance (excluding Acari). However, in some taxonomic groups such as Araneae, Diptera, Thysanoptera and Psocoptera, immature individuals outnumbered adults. Lepidoptera was the only order that was represented primarily by larvae (except for three adult specimens). The largest proportion of immature invertebrates was collected in January (47.5%), a result driven by high numbers of juveniles in ant colonies at Karamea. In the April 2004 and 2005 collection periods, an average 16.6% of immature individuals was recorded, while the lowest proportions of immature specimens were recorded in July (9.8%) and October (9.5%) (Fig. 3).

Communities at both sites included all feeding guilds. Of the total number of species detected, c. 85% could be assigned to guilds. Most species represented herbivores (chewers 18.6% and sap feeders 15.8%), followed by predators (23.6%), scavengers (13.6%), parasitoids (10.8%) and epiphyte grazers (10.3%). Epiphyte grazers, exclusively Collembola, dominated communities in abundance (49.8%) followed by ants (32.7%), herbivores (5.6%), predators (4.4%) and scavengers (3.2%). Parasitoids were represented by 0.27% of the total number of species.

There was generally little change in overall guild composition across seasons. However, parasitoids ($t = 1.8$; $P < 0.05$) and sap feeders ($t = 2.4$; $P < 0.05$) were represented by significantly more species in April compared with July and October, while the species richness of the other guilds remained similar across sampling periods (Fig. 4A). All guilds were most abundant in January (sap feeder, epiphyte grazers) or April (ants, chewers, predators, scavengers), except for parasitoids which peaked in July. The

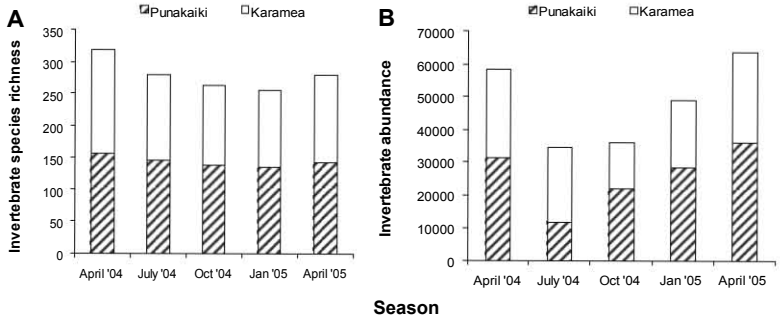

Fig. 2 Seasonal variation in species richness (**A**) and abundance (**B**) of canopy invertebrates collected from 120 epiphyte mats in northern rata at two study sites.

largest fluctuation in abundance was recorded for ants; 52.4% of the total number of ants were collected in April 2004 compared with 6.6–8.7% in the July to January sampling periods (Fig. 4B). However, the number of epiphyte samples containing large ant colonies of more than 100 individuals was also higher in April 2004 than for any other time of year.

Noteworthy discoveries

This study discovered several undescribed species in the epiphyte mat habitats. Of particular interest was the discovery of a new genus of felt scale, *Affeldococcus kathrinae*, (Hemiptera: Eriococcidae) (Henderson 2007) and new species of the genera *Acrochordonus* and *Chorizococcus* (both Hemiptera: Pseudococcidae), *Poropeza* "near" *dacrydii* (Hemiptera: Coccidae). Further new discoveries included the potentially new Yponomeutoidae sp. (Lepidoptera), two new species of the Diplopod family Dalodesmidae including a *Tongodesmus* species, and a spider species from an undescribed family. The total number of new species is estimated to be much higher, given that the majority of specimens could be identified only to genus or family level.

Apart from new species, this study also recorded extended geographical and habitat ranges for various known species. First arboreal distributions were recorded for the Lepidopteran genus *Mallobathra* (Psychidae), and for the species *Cryptaspasma querula* (Tortricidae), wingless Sciaridae (Diptera) and the rare beetle *Paracorneolabium brouni* (Coleoptera: Staphylinidae). The Hemipteran species *Newsteadia gullanae* (Ortheziidae), described from

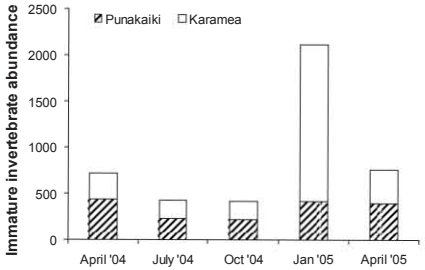

Fig. 3 Seasonal variation in the abundance of immature invertebrates collected from 120 epiphyte mats in the canopy of northern rata at two study sites.

Australia, has so far been found only in this study and in a similar canopy study of epiphyte mats in rimu (Affeld 2002).

One of the most surprising finds was the discovery of large arboreal ant colonies. Several hundreds to thousands of individuals of the species *Prolasius advena* were found in 25 out of the 120 samples collected (21%), and lower numbers of ants (<100 individuals) in 82% of all samples. To our knowledge this is the first record for New Zealand of arboreal colonies of an ant species that until now has been described as ground nesting (Don 2007). Ants are highly abundant in the canopies of many tropical rain forests (Huxley 1980; Stork 1987; Floren & Linsenmair 2005) where they often form close associations with honeydew secreting Hemipteran insects (Bach

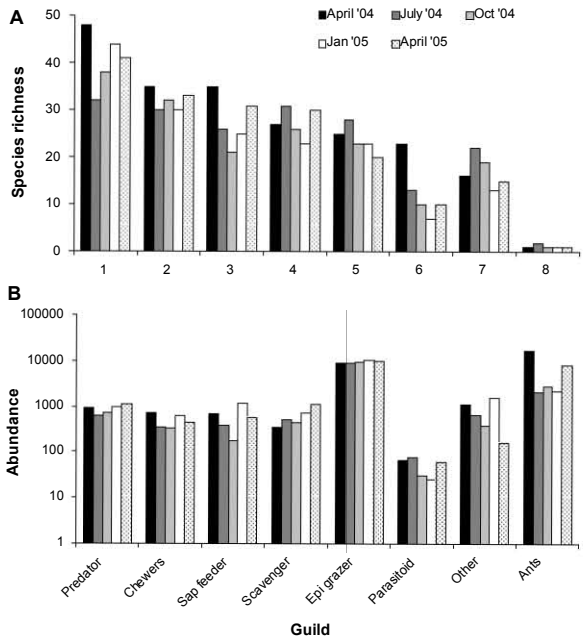

Fig. 4 Seasonal variation in species richness (**A**) and abundance (**B**) of invertebrate guilds collected from 120 epiphyte mats in the canopy of northern rata at two study sites combined.

1991; Davidson 1997). Of the species present in this study *Prolasius advena* is known to tend *Poropeza dacrydii* (Hemiptera: Coccoidea) (Hodgson & Henderson 2000) and possibly *Poropeza cologabata* (RH pers. obs.) but we found no correlation between ant abundance and *P. dacrydii* ($r = 0.026$; $P > 0.05$), or *P. cologabata* ($r = 0.032$; $P > 0.05$), or presence/absence between these groups.

Exotic species

This study confirmed records of the following introduced species: *Limothrips cerealium* (Thysanoptera: Thripidae), the nectar and pollen feeding beetles ?*Anthrenocerus australis* and *Reesa vespulae* (both Coleoptera: Dermestidae), the fungal feeding *Ephistemus globulus* and a *Cryptophagus* sp. (ST pers. obs.) (both Coleoptera: Cryptophagidae) and the two Diptera species *Anthomyia punctipennis* (Anthomyiidae) and *Lonchoptera furcata* (Lonchopteridae). All these species were represented by only one specimen, or one specimen per site for *L. cerealium*, indicating that they may have been incidentals. The native species *Thrips obscuratus* (Thysanoptera: Thripidae) is known to cause destruction in flower and crop production (LM pers. obs.) and was highly abundant in Punakaiki. This species swarms at certain times of year, but its impact on native biodiversity is unknown.

DISCUSSION

The canopy of northern rata supports a highly diverse and abundant invertebrate fauna, that is diverse both taxonomically and functionally. Considering (1) that this study focused exclusively on one habitat type and one tree species, (2) that the sampling method is likely to have missed many flying insects and other highly mobile invertebrates, and (3) that it was impossible to identify the larval stages of many invertebrates, this inventory could list only a portion of the true invertebrate biodiversity in canopy habitats of mixed podocarp-broadleaf forests. In particular, the number of species in orders such as Acari,

Diptera, Hymenoptera and Lepidoptera are likely to be grossly underrepresented in this inventory.

Abundant invertebrate faunas have been recorded in other canopy habitats, including phytotelmata and suspended soils (Greeney 2001; Wardle et al. 2003; Derraik & Heath 2005; Lindo & Winchester 2006), and therefore should be included in future canopy studies. Similarly, a range of tree species should be sampled to fully understand how much canopy invertebrates contribute to overall biodiversity in New Zealand's rain forests and how they are distributed. For example, Didham (1997), recorded 373 Diptera species in a mixed podocarp-broadleaf forest using flight interception traps compared to the 19 species collected in this study. Consequently, a combination of different sampling methods would benefit future inventory studies.

The composition of invertebrate communities in this study was similar for both study sites and was characterised, in terms of abundance, by the dominance of Acari, Collembola and ants. The proportionally high contribution of these taxonomic groups to overall community composition reflects the ranking of dominant groups, with respect to species abundance and richness, in many forest systems worldwide (Paoletti et al. 1991; Ellwood et al. 2002; Wardle et al. 2003; Yanoviak et al. 2004; Lindo & Winchester 2006). The high frequency and, in some cases, vast abundance of ants in this study, however, was very unusual for a temperate rain forest and more reminiscent of canopy communities in tropical forests.

With regards to feeding guilds, the high abundance of Collembolan epiphyte grazers is typical of habitats with a substantial humus component (Wardle et al. 2003), whereas the high species richness of herbivores may reflect the diversity in plant-derived resources provided by the epiphyte mats. While variation in guild composition may reflect differences in habitat type, it also raises the issue of guild assignment, particularly for species whose feeding habitats are unknown, as is often the case in highly diverse canopy habitats, including this one. In some cases, guild assignments of such species were inferred from known feeding habits of closely related species or entire taxa, such as Collembola and Araneae. In other instances, species, including mites and immature specimens, were excluded from species richness and guild analysis, because their biology is poorly understood and they cover a wide range of feeding habits. Yet other species, including those that fall into more than one guild, were placed into the "others" guild. Either of these guild assignments for species with unknown feeding habit may have resulted in the misrepresentation of the abundance of some guilds. While the results presented here for feeding guilds offer an incomplete picture of the invertebrate communities in this study, they nevertheless give a preliminary indication of functional community structure, an aspect that is often neglected in invertebrate community studies.

Seasonal fluctuations in invertebrate abundance were relatively minor throughout the year, particularly at Karamea. However, the much lower number of invertebrates recorded at Punakaiki in winter is possibly a reflection of the cooler winter temperatures experienced at this site compared to Karamea. Small differences in abundance were observed for the various guilds among seasons and between sites. Seasonal changes in community composition probably reflected patterns related to the life history of specific invertebrate groups or species, the seasonal availability of different foods, and seasonal weather patterns. Seasonal variation in ant abundance, however, was apparently driven by chance encounters of entire colonies during sampling, correlated with their highly clumped distribution.

While sample collection from canopy habitats can be labour intensive and logistically challenging, the true challenge lies in the sorting and identification of invertebrate specimens. Taxa such as Hymenoptera, Diptera, Araneae, Collembola and Acari were particularly difficult. The high proportion of undescribed species and lack of taxonomic keys and expertise for these groups made identification beyond family level largely impossible. These results reflect the poor knowledge of much of the New Zealand invertebrate fauna (Emberson 1994; Halloy et al. 2001), but also show that proportionately fewer insect and non-insect species of canopy habitats have been described compared with those of ground-based habitats. Taxonomists and researchers face immense tasks and challenges when incorporating invertebrates into field projects. Despite such limitations, studies of canopy invertebrates in New Zealand's rain forests offer much scope for providing new information on invertebrate species' distributions and exploring different aspects of their ecology and involvement in ecosystem processes.

An important aspect of canopy invertebrate studies in indigenous forest ecosystems is that of assessing the risk of invasion of these habitats by exotic species, and the vulnerability of native invertebrates to such threats. Seven exotic insect species were identified in this study, but because each was collected in low numbers, it is unlikely that they pose an immediate threat to native canopy communities.

Their origins are unclear, but it is possible that they came from areas with human activity (farming, horticulture, gardening) located within 10 km of the study sites. Although this study has shown that exotic invertebrate species can penetrate deep into this native forest system, the relative lack of exotic species in the canopy was surprising. Canopy habitats provide potentially suitable conditions for exotic species that could facilitate their establishment in the future. Of particular concern are threats from invasive species with a well documented detrimental track record elsewhere. Some of these could potentially establish in New Zealand, such as the yellow crazy ant that by its activities has modified the character of forests on Christmas Island (O'Dowd et al. 2003).

CONCLUSIONS

Many uncertainties remain concerning the consequences of exotic invasion for native ecosystems, especially for ecosystems that are poorly understood like the forest canopy. Baseline data is therefore needed for any future monitoring programmes to not only detect the presence of potentially harmful species at the early stages, but also to assess their impact on potential keystone canopy species and the implications for entire communities. However, that requires a good understanding of the species inhabiting forest canopies, their ecologies and interactions, compositional and distributional patterns in canopy communities, and species involvement in ecosystem processes.

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Appendix Species list of invertebrates collected in 120 epiphyte mats on northern rata at two study sites, Punakaiki and Karamea between April 2004 and April 2005. Shown are the frequencies of each species occurrence at each of the two study sites and across seasons. The species status is indicated as E = endemic, I = introduced, C = cosmopolitan, N = native.

	Site		Sampling time							
	Punakaiki	Karamea	Apr 04	Jul 04	Oct 04	Jan 04	Apr 05	Guild	Status	ID
Coleoptera										
Anobiidae										
Ptinus speciosus	–	1	1	–	–	–	–	Other	E	S. Thorpe
Brentidae										
Neocyba metrosideros	1	1	1	–	–	–	1	Chewer	E	S. Thorpe
Byrrhidae										
Byrrhidae sp.	1	–	–	1	–	–	–	Chewer	E	S. Thorpe
Cantharidae										
?Cantharidae sp.	1	–	–	–	–	–	1	Predator		S. Thorpe
Carabidae										
Amarotypus edwardsi	1	1	–	–	–	1	1	Predator	E	S. Thorpe
Mecodema ducale	1	1	1	–	–	–	1	Predator	E	S. Thorpe
Platynus macropterus	–	1	–	–	1	–	–	Predator	E	S. Thorpe
Cerambycidae										
?*Tenebrosoma* sp.1	–	1	1	–	–	–	–	Chewer	E	S. Thorpe
?*Tenebrosoma* sp.2	–	1	1	–	–	–	–	Chewer	E	S. Thorpe
Chrysomelidae										
?*Caccomolpus* sp.	3	1	–	2	1	–	1	Chewer	E	S. Thorpe
Coccinellidae										
?*Adoxellus* sp.	–	6	2	3	–	–	1	Predator	E	S. Thorpe
Rhyzobius "small, dark"	12	17	4	10	6	7	2	Predator	E	S. Thorpe
Corylophidae										
Holopsis sp.1	1	2	1	–	–	1	1	Fungivore	E	S. Thorpe
Holopsis sp.2	–	1	–	–	–		1	Fungivore		S. Thorpe
Sericoderus sp.	3	1	–	–	–	2	2	Fungivore	E	S. Thorpe
Cryptophagidae										
Cryptophagus sp.	1	–	–	–	1	–	–	Fungivore	I	S. Thorpe
Ephistemus globulus	1	–	–	–	–	1	–	Fungivore	I	S. Thorpe
?*Micrambina* sp.	4	–	–	2	1	–	1	Fungivore	E	S. Thorpe
Paratomaria sp.	1	2	2	1	–	–	–	Fungivore	E	S. Thorpe
Curculionidae										
Andracalles sp.	24	17	8	6	13	5	9	Chewer	E	S. Thorpe
Bradypatae sp.	–	1	–	–	–	–	1	Herbivore	E	S. Thorpe
Cossoninae sp.1	–	2	1	–	–	1	–	Chewer	E	S. Thorpe
Curculionidae sp.	–	1	1	–	–	–	–	Chewer		S. Thorpe
Euophryum sp.	1	–	–	–	–	1	–	Chewer	E	S. Thorpe

Geochus sp.	1	7	2	4	–	–	2	Chewer	E	S. Thorpe
?*Metacalles* sp.	20	10	7	6	6	6	5	Chewer	E	S. Thorpe
?*Microcryptorhynchus* sp.	19	11	11	5	4	9	1	Chewer	E	S. Thorpe
Neomycta rubra	8	–	2	3	1	–	2	Chewer	E	S. Thorpe
Nestrius sp.	–	2	–	–	–	–	2	Herbivore	E	S. Thorpe
Pachyderris sp.	–	1	–	1	–	–	–	Chewer	E	S. Thorpe
Reyesiella sp.	–	2	1	–	1	–	–	Chewer	E	S. Thorpe
?*Trinodicalles* sp.	3	–	–	1	2	–	–	Chewer	E	S. Thorpe
Zeacalles sp.	–	1	–	–	–	–	1	Herbivore	E	S. Thorpe
Cyclaxyridae										
Cyclaxyra sp.	2	–	–	–	2	–	–	Fungivore	E	S. Thorpe
Dermestidae										
?*Anthrenocerus australis*	1	–	–	–	1	–	–	Chewer	?I	S. Thorpe
Reesa vespulae	–	1	–	–	–	1	–	Chewer	I	S. Thorpe
Endomychidae										
Holoparamecus sp.	0	2	–	–	–	–	2	Fungivore		S. Thorpe
Latridiidae										
Latridiidae sp.	–	4	–	2	2	–	–	Fungivore	E	S. Thorpe
Melandryidae										
Melandryidae sp.	–	1	–	–	1	–	–	Other	E	S. Thorpe
Nemonychidae										
Rhinorhynchus rufulus	2	–	–	–	1	–	1	Chewer	E	S. Thorpe
Nitidulidae										
?*Epuraea* sp.	–	1	–	–	1	–	–	Sap feeder	E	S. Thorpe
Hisparonia hystrix	1	–	–	–	–	1	–	Sap feeder	E	S. Thorpe
Ptiliidae										
?*Nellosana* sp.	1	2	–	–	–	–	3	Fungivore		S. Thorpe
Scirtidae										
Amplectopus sp.	1	–	–	–	–	1	–	Chewer	E	S. Thorpe
Scirtidae sp.	4	–	–	–	3	1	–	Chewer	E	S. Thorpe
Scydmaenidae										
Scydmaenidae sp. mix	–	2	2	–	–	–	–	Predator	E	S. Thorpe
?*Microscydmus* sp.	34	25	12	8	9	16	14	Predator	E	S. Thorpe
Staphylinidae										
Aleocharinae sp.	3	3	2	1	1	2	–	Predator	E	K. Thayer
Eupines sp.	–	1	–	–	–	1	–	Predator	E	S. Thorpe
Euplectine sp.1	25	21	7	8	11	10	10	Predator	E	S. Thorpe
Euplectine sp.2	3	8	7	1	–	1	2	Predator	E	S. Thorpe
Euplectine sp.3	16	13	2	8	8	5	6	Predator	E	S. Thorpe
Euplectine sp.4	1	–	–	–	1	–	–	Predator	E	S. Thorpe
Euplectine sp.5	1	1	–	–	–	1	1	Predator	E	S. Thorpe
Euplectine sp.6	–	1	–	–	–	1	–	Predator	E	S. Thorpe

(*continued*)

Appendix (*contd*)

	Site		Sampling time							
	Punakaiki	Karamea	Apr 04	Jul 04	Oct 04	Jan 04	Apr 05	Guild	Status	ID
Hamotulus sp.	5	1	1	2	1	1	1	Predator		S. Thorpe
Mesoaesthetus sp.	4	2	–	1	2	2	1	Predator	E	K. Thayer
Paratorchus sp.1	1	7	3	2	2	–	1	Other	E	K. Thayer
Paratorchus sp.2	1	4	1	1	2	1	–	Other	E	K. Thayer
?*Sagola* sp.1	2	1	–	–	1	2	–	Predator	E	S. Thorpe
?*Sagola* sp.2	1	3	1	–	2	1	–	Predator	E	S. Thorpe
?*Sagola* mix	6	4	–	5	2	1	2	Predator	E	S. Thorpe
Paracorneolabium brouni	7	3	1	1	3	1	4	Other	E	S. Thorpe
Tenebrionidae										
Archaeoglenes costipennis	–	1	1	–	–	–	–	Scavenger	E	S. Thorpe
Artystona sp.	–	1	–	–	–	–	1	Lichenfeeder		S. Thorpe
?*Cerodolus* sp.	5	2	2	1	1	2	1	Lichen feeder	E	S. Thorpe
Zopheridae										
Ablabus sp.	–	1	–	–	–	1	–	Fungivore	E	S. Thorpe
?*Heterargus* sp.	–	1	1	–	–	–	–	Fungivore	E	S. Thorpe
Notocoxelus sp.	3	3	1	1	2	1	1	Fungivore	E	S. Thorpe
Pycnomerus sp.1	–	1	–	–	–	1	–	Fungivore	E	S. Thorpe
Indet.										
Indet. spp. [immature]	58	54	19	20	24	22	27	Other		
Hymenoptera										
Aphelinidae										
Aphelinidae sp.1	2	–	1	1	–	–	–	Parasitoid		J. Early
?Aphelinidae sp.2	1	–	–	1	–	–	–	Parasitoid		J. Early
Aphelinidae sp.3	7	1	–	2	3	1	2	Parasitoid		J. Early
Braconidae										
Braconidae sp.	1	–	–	1	–	–	–	Parasitoid		J. Early
Ceraphronidae										
Ceraphronidae sp.1	1	–	1	–	–	–	–	Parasitoid		J. Early
Ceraphronidae sp.2	1	–	1	–	–	–	–	Parasitoid		J. Early
Ceraphronidae sp.3	1	1	2	–	–	–	–	Parasitoid		J. Early
Ceraphronidae sp.4	1	–	1	–	–	–	–	Parasitoid		J. Early
Cynipoidea										
Cynipoidea sp.1	1	–	–	–	1	–	–	Parasitoid		J. Early
Cynipoidea sp.2	1	–	–	–	–	–	1	Parasitoid		J. Early
Diapriidae										
Betyla ?*eupepla*	1	–	–	1	–	–	–	Parasitoid	E	J. Early
Betyla wahine	2	–	1	1	–	–	–	Parasitoid	E	J. Early
Entomacis sp.	1	–	–	–	1	–	–	Parasitoid	E	J. Early

Pantolytomyia taurangi	1	–	–	1	–	–	–	Parasitoid	E	J. Early
Stylaclista sp.	1	4	1	1	–	3	–	Parasitoid	E	J. Early
Trichopria sp.	–	2	–	–	2	–	–	Parasitoid		J. Early
Encyrtidae										
Encyrtidae sp.	–	1	1	–	–	–	–	Parasitoid		J. Early
Eulophidae										
Eulophidae sp.	–	1	1	–	–	–	–	Parasitoid		J. Early
Formicidae										
Discothyrea antarctica	1	–	1	–	–	–	–	Ant	E	K. Affeld
Huberia brounii	–	1	–	1	–	–	–	Ant	E	K. Affeld
Prolasius advena	52	46	24	14	20	21	19	Ant	E	K. Affeld
Ichneumonidae										
Ichneumonidae sp.1	–	1	–	–	1	–	–	Parasitoid		J. Early
Ichneumonidae sp.2	1	1	–	–	2	–	–	Parasitoid		J. Early
Megaspilidae										
Lagynodes gastroleius	10	1	5	–	–	2	4	Parasitoid		J. Early
Megaspilidae sp.1	8	–	4	4	–	–	–	Parasitoid		J. Early
Megaspilidae sp.2	1	1	2	–	–	–	–	Parasitoid		J. Early
Megaspilidae sp.3	–	1	1	–	–	–	–	Parasitoid		J. Early
Megaspilidae sp.4	1	–	1	–	–	–	–	Parasitoid		J. Early
Megaspilidae sp.5	–	4	–	–	1	–	3	Parasitoid		J. Early
Mymaridae										
Cleruchus sp.	–	1	1	–	–	–	–	Parasitoid		J. Early
Mymaridae sp.1	–	2	1	1	–	–	–	Parasitoid		J. Early
Mymaridae sp.2	–	1	1	–	–	–	–	Parasitoid		J. Early
Mymaridae sp.3	2	–	2	–	–	–	–	Parasitoid		J. Early
Mymaridae sp.4	7	7	–	3	2	2	7	Parasitoid		J. Early
?*Neserythmelus* sp.	2	1	–	3	–	–	–	Parasitoid		J. Early
Platygastridae										
Errolium sp.	4	2	2	–	–	2	2	Parasitoid		J. Early
Platygastridae sp.1	2	–	1	–	–	–	1	Parasitoid		J. Early
Platygastridae sp.2	1	–	1					Parasitoid		J. Early
Platygastridae sp.3	–	1	–	–	–	1	–	Parasitoid		J. Early
Scelionidae										
Baeus sp.	–	1	–	–	1	–	–	Parasitoid		J. Early
Idris sp.1	1	1	1	1	–	–	–	Parasitoid		J. Early
Idris sp.2	2	–	–	–	–	1	1	Parasitoid		J. Early
Mirobaeus sp.	–	1	–	–	–	–	1	Parasitoid		J. Early
Trichogrammatidae										
Trichogrammatidae sp.	2	–	–	–	–	–	2	Parasitoid		J. Early
Indet.										
Indet. sp.	1	1	–	–	2	–	–	Parasitoid		J. Early
Indet. spp. [immature]	1	3	–	–	–	4	–	Other		J. Early

(*continued*)

Appendix (*contd*)

	Site		Sampling time							
	Punakaiki	Karamea	Apr 04	Jul 04	Oct 04	Jan 04	Apr 05	Guild	Status	ID
Hemiptera										
Heteroptera										
Aleyrodidae										
white fly	2	–	–	–	1	–	1	Sap feeder		J. Early
Aphididae										
Aphididae sp.1	–	1	1	–	–	–	–	Sap feeder		K. Affeld
Aphididae sp.2	3	–	2	–	–	–	1	Sap feeder		K. Affeld
Aphididae sp.3	2	1	1	1	1	–	–	Sap feeder		K. Affeld
Aphididae sp.4	1	–	1	–	–	–	–	Sap feeder		K. Affeld
Aphididae sp. [immature]	3	8	3	6	–	1	1	Sap feeder		K. Affeld
Cicadellidae										
Cicadellidae sp.1	2	–	1	–	–	–	1	Sap feeder		K. Affeld
Cicadellidae sp.2	–	1	1	–	–	–	–	Sap feeder		K. Affeld
Cicadellidae sp.3	1	1	2	–	–	–	–	Sap feeder		K. Affeld
Cicadellidae sp.4	1	–	–	1	–	–	–	Sap feeder		K. Affeld
Cicadellidae sp. [immature]	5	2	2	1	1	–	3	Sap feeder		K. Affeld
Cicadidae										
Amphipsalta zelandica	–	1	–	–	–	–	1	Sap feeder		K. Affeld
Enicocephalidae										
Enicocephalidae sp.	1	–	–	1	–	–	–	Sap feeder		K. Affeld
Psyllidae										
Psyllidae sp.	–	1	–	1	–	–	–	Sap feeder		R. Henderson
Rhyparochromidae										
Rhyparochromidae sp.	12	16	7	4	9	3	5	Sap feeder		K. Affeld
Rhyparochromidae sp. [immature]	17	26	12	7	12	4	8	Sap feeder		K. Affeld
Homoptera										
Coccidae										
Coccidae sp.	1	1	1	1	–	–	–	Sap feeder		R. Henderson
"long setae" sp.	3	1	4	–	–	–	–	Sap feeder		R. Henderson
"neonate" sp.	2	–	1	–	–	1	–	Sap feeder		R. Henderson
Plumichiton flavus	–	1	–	1	–	–	–	Sap feeder		R. Henderson
Poropeza "near" *dacrydii*/?new	1	1	–	–	1	–	1	Sap feeder		R. Henderson
Poropeza cologabata	4	–	2	–	–	–	2	Sap feeder		R. Henderson
"short setae" sp.	1	–	1	–	–	–	–	Sap feeder		R. Henderson
Coelostomidiidae										
Coelostomidia montana	1	–	1	–	–	–	–	Sap feeder		R. Henderson

Diaspididae									
?*Anoplaspis* sp.	4	–	–	1	3	–	–	Sap feeder	R. Henderson
Diaspididae sp.	4	2	1	–	1	2	2	Sap feeder	R. Henderson
Leucaspis sp.	4	2	3	–	–	1	2	Sap feeder	R. Henderson
Eriococcidae									
Affeldococcus kathrinae	4	3	3	–	–	3	1	Sap feeder	R. Henderson
Eriochiton sp.	1	2	1	1	–	–	1	Sap feeder	R. Henderson
Eriochiton spinosus	–	3	–	1	1	1	–	Sap feeder	R. Henderson
Eriococcus abditus	5	6	2	2	4	2	1	Sap feeder	R. Henderson
Eriococcus albatus	–	1	2	6	1	1	–	Sap feeder	R. Henderson
?*Eriococcus albatus*	–	1	–	–	–	1	–	Sap feeder	R. Henderson
Eriococcus "apterous" sp.	2	–	–	–	–	2	–	Sap feeder	R. Henderson
Eriococcus "apterous" ?*elytranthae*	1	–	–	–	–	1	–	Sap feeder	R. Henderson
Eriococcus elytranthae	11	–	4	1	–	4	2	Sap feeder	R. Henderson
Eriococcus rata	16	13	3	7	4	9	6	Sap feeder	R. Henderson
Eriococcus ?*rata*	–	5	–	–	–	–	5	Sap feeder	R. Henderson
Eriococcus sp. 1	4	3	3	1	1	–	2	Sap feeder	R. Henderson
Eriococcus sp. 2	3	2	1	–	–	2	2	Sap feeder	R. Henderson
Ortheziidae									
Ortheziidae sp.	3	–	3	–	–	–	–	Sap feeder	R. Henderson
Newsteadia gullanae	1	1	–	1	1	–	–	Sap feeder	R. Henderson
Newsteadia myersi	12	–	3	3	1	2	3	Sap feeder	R. Henderson
Newsteadia sp.	8	2	2	–	2	2	4	Sap feeder	R. Henderson
Phenacoleachiidae									
Phenacoleachia sp.	3	3	–	–	3	2	1	Sap feeder	R. Henderson
Phenacoleachia zealandica	2	1	2	1	–	–	–	Sap feeder	R. Henderson
Pseudococcidae									
?*Balanococcus* sp.	2	1	–	1	–	–	2	Sap feeder	R. Henderson
?*Chorizococcus* sp./new	1	–	–	–	–	–	1	Sap feeder	R. Henderson
Laminicoccus asteliae	–	2	1	–	1	–	–	Sap feeder	R. Henderson
"Mealybug" sp.	4	15	3	1	3	7	5	Sap feeder	R. Henderson
Pseudococcidae "neonate" sp.	1	–	–	–	–	1	–	Sap feeder	R. Henderson
Pseudococcidae "apterous" sp.	1	1	1	–	–	–	1	Sap feeder	R. Henderson
Pseudococcidae sp. 1	1	6	7	–	–	–	–	Sap feeder	R. Henderson
Pseudococcidae sp. 2	6	2	–	3	3	1	1	Sap feeder	R. Henderson
Paracoccus ?*glaucus*	1	–	–	–	–	–	1	Sap feeder	R. Henderson
Paracoccus sp.	6	5	6	1	–	1	3	Sap feeder	R. Henderson
?*Paracoccus* sp.	1	1	–	1	–	1	–	Sap feeder	R. Henderson
Ripersiella deboerae	2	5	–	1	2	2	2	Sap feeder	R. Henderson
Ripersiella puhiensis	5	3	1	1	2	2	2	Sap feeder	R. Henderson
Ripersiella ?*puhiensis*	–	1	–	–	–	–	1	Sap feeder	R. Henderson
Ripersiella sp.	–	3	3	–	–	–	–	Sap feeder	R. Henderson

(*continued*)

Appendix (*contd*)

	Site		Sampling time							
	Punakaiki	Karamea	Apr 04	Jul 04	Oct 04	Jan 04	Apr 05	Guild	Status	ID
Ripersiella sp./apterous males	2	–	–	–	–	–	2	Sap feeder		R. Henderson
Indet.	1	–	–	1	–	–	–	Sap feeder		R. Henderson
Lepidoptera										
Carposinidae										
Heterocrossa ?*epomiana* [immature]	–	1	1	–	–	–	–	Chewer		L. Clunie & J. Dugdale
Crambidae										
Glaucocharis sp. [immature]	1	–	–	–	–	–	1	Herbivore		L. Clunie
Geometridae										
Geometridae sp. [immature]	4	–	1	–	1	2	–	Chewer		L. Clunie
?Nepticulidae										
?Nepticulidae sp. [immature]	1	–	1	–	–	–	–	Chewer		L. Clunie
Noctuidae										
Plusiinae sp. [immature]	–	1	1	–	–	–	–	Other		L. Clunie
Oecophoridae										
Atomotricha sp. [immature]	2	1	1	–	1	1	–	Chewer		L. Clunie
Gymnobathra sp. [immature]	2	2	2	–	2	–	–	Chewer		L. Clunie & J. Dugdale
Gymnobathra "spotty" [immature]	–	1	–	–	1	–	–	Chewer		L. Clunie
?*Gymnobathra* sp. [immature]	1	–	1	–	–	–	–	Chewer		L. Clunie & J. Dugdale
?*Leptocroca* sp. [immature]	1	–	–	–	–	1	–	Chewer		L. Clunie
Oecophoridae sp. [immature]	3	4	–	–	–	3	4	Chewer		L. Clunie & J. Dugdale
?Oecophoridae sp. [immature]	1	6	2	3	–	1	1	Chewer		L. Clunie
Thamnosara sp. [immature]	–	2	–	–	1	–	1	Chewer		L. Clunie
?*Thamnosara* sp. [immature]	1	–	–	–	–	–	1	Chewer		L. Clunie
Tingena sp. [immature]	34	22	12	10	13	8	13	Chewer		L. Clunie & J. Dugdale
Tingena "spotty" [immature]	–	5	–	1	2	1	1	Chewer		L. Clunie
?*Tingena* sp. [immature]	3	–	1	1	–	–	1	Chewer		L. Clunie
Trachypepla sp. [immature]	1	1	2	–	–	–	–	Other		L. Clunie
Psychidae										
Grypotheca sp. [immature]	5	2	–	2	3	2	–	Chewer		L. Clunie
Mallobathra sp. [immature]	7	4	1	1	2	4	3	Chewer		L. Clunie & J. Dugdale
?*Mallobathra* sp. [immature]	1	–	–	1	–	–	–	Chewer		L. Clunie
Psychidae sp. [immature]	9	5	4	2	2	2	4	Chewer		L. Clunie
?Psychidae sp. [immature]	2	–	–	2	–	–	–	Chewer		L. Clunie
Reductoderces sp. [immature]	22	11	6	3	10	8	6	Chewer		L. Clunie & J. Dugdale
?*Reductoderces* sp. [immature]	1	–	1	–	–	–	–	Chewer		L. Clunie
Scoriodyta sp. [immature]	6	1	2	1	2	–	2	Chewer		L. Clunie & J. Dugdale