Morphology, Phylogeography and Drumming Behaviour of a New Zealand Ground Weta, *Hemiandrus pallitarsis*.

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Abstract

Species are one of the fundamental components of biology and the accurate delimitation of species is important in evolutionary, systematic and ecological studies, yet there is still confusion over how species can be recognised. Examining different characters allows multiple lines of evidence for successful and accurate species delimitation and identification. In this thesis, morphological, genetic and behavioural variation is investigated within an endemic species of ground weta, *Hemiandrus pallitarsis*, in the North Island, New Zealand. Twelve morphological characters were measured, and mitochondrial cytochrome oxidase I DNA sequences were analysed from populations across the distributional range of *H. pallitarsis.* Both methods provide no evidence of a species complex within *H. pallitarsis*. Instead, the morphometric results suggest females are significantly larger than males, and ground weta in Palmerston North are significantly smaller than weta further north. Additionally, genetic analyses found substantial population structuring, large genetic distances, and an historical south to north pattern of movement in the North Island. The pattern of vibratory drumming behaviour followed that predicted by morphology and geographic proximity – drumming signals were more similar between geographically close populations and did not match the patterns of genetic isolation. Overall, this thesis was able to show that *H. pallitarsis* is morphologically, genetically and behaviourally variable across the North Island.

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Chapter 1: Thesis Introduction

1.1 Species concepts

Since before Darwin, there has been debate and disagreement concerning the meaning of the word 'species'. Darwin himself recognised the fact that there were multiple definitions used by naturalists, but that everyone had the same general concept of what a species was (Darwin, 1859). Over the last 60 years, there has been a plethora of species concepts formulated, such as the Biological species concept (Mayr, 1942), the Recognition species concept (Paterson, 1985), the Ecological species concept (Van Valen, 1976), and the Evolutionary species concept (Simpson, 1951), to name just a few. In fact, there are at least 24 different named species concepts (Mayden, 1997). Much of the argument surrounding the debate has been over theoretical concepts versus methodological ones, that is, concepts with specific species identification criteria. Nevertheless, De Queiroz (2007) suggests there is a common element among all contemporary concepts and proposes a unified species concept in which species are "…separately evolving metapopulation lineages, or more specifically, segments of such lineages" (p. 880-881). This concept, De Queiroz points out, separates the issue of species concept from species delimitation, where boundaries and numbers of species are inferred. Therefore, the general consensus seems to be that members of a species have an evolutionary history more similar to members in the same species than they do with other species (Hey, 2006).

Despite the controversy surrounding species concepts, some authors have separated species identification and delimitation from the more conceptual aspect and have focused solely on identification (operational) criteria (e.g. Sites & Marshall, 2003; Winston, 1999). However, the operational criteria used in species delimitation have also come under scrutiny (Wiens & Servedio, 2000). DNA sequence data is now commonly used to infer species boundaries (Besansky *et al.*, 2003; Puorto *et al.*, 2001), although most species continue to be identified based on morphological descriptions. Indeed, several authors have stressed the importance of examining multiple different characters in order to gain evidence for the existence of separate species (e.g. De Queiroz, 2007; Roe & Sperling, 2007). The concordance of characters (e.g.

morphological, genetic, behavioural) can form crucial evidence on which species status decisions are made. Accordingly, this thesis sets out to document the degree of morphological, genetic and behavioural variation within an endemic speices of ground weta, *Hemiandrus pallitarsis*, in NI, New Zealand.

1.2 New Zealand ground weta

The Southern Hemispheric group of ensiferan Orthoptera, which form a major group in New Zealand, are the weta, or king crickets. Previous taxonomic classification placed all New Zealand weta into the family Stenopelmatidae (Hutton, 1896), which was divided into two sub-families, *Anostominae* (with pads on lower surface of tarsi) and *Dolichopodinae* (without any pads). Johns (1997), however, raised the sub-family *Anostominae* to full family status – Anostostomatidae, and suggested weta in this family could be distinguished from Stenopelmatidae based on features of the fastigium, coxae, foretibia, metasternum and hindfemur. There are four groups of Anostostomatid weta in New Zealand: ground weta (*Hemiandrus*), tree weta (*Hemideina*), giant weta (*Deinacrida*), and tusked weta (*Motuweta*, *Anisoura*); of these four groups, ground weta are the most speciose.

Ground weta are nocturnal flightless insects, ranging in size from 12 to 45 mm long. During the day they seal themselves in burrows in the ground, and at night they feed primarily on other invertebrates, but also eat fruit and seeds. Like cave weta (Orthoptera: Rhaphidophoridae), they do not possess tibial tympanal organs and so presumably are deaf to airborne sound, they do however have abdominal stridulatory structures, usually reduced to conical pegs or small rounded knobs on the first two or three tergites (Field, 2001). Ground weta inhabit a wide variety of environments including urban gardens, grasslands, shrublands and forests, and are found throughout New Zealand, from the subantarctic islands to Northland.

There are currently nine named and more than 28 undescribed *Hemiandrus* species in New Zealand and several species in Australia (Jewell, 2007; Johns, 1997, 2001). The species of interest in this study was *Hemiandrus pallitarsis*, a ground weta with a characteristically short ovipositor and modified sixth abdominal sternite. There are seven species of short ovipositor weta in New Zealand (two described and five undescribed) (Johns, 2001). The majority of these species are found only in the South

Island: *Hemiandrus* "turgidulus" is found in the Cromwell river sand dunes, an area protected for the Cromwell chafer beetle (*Prondontria lewisii*); *H.* "horomaka" is endemic to Banks Peninsula; *H.* "onokis" occurs throughout the North Canterbury area; *H.* "promontorius" is found only at Cape Campbell and Marfell's Beach; and *H.* "vicinus" is found in the Marlborough Sounds area. There are two short ovipositor species in the NI which are sympatric: *H. bilobatus* occurs in the Wellington region as far north as Paraparamu and is found on Mana Island, Matiu/Somes Island and the Brothers Island on the south side of the Cook Strait; and *H. pallitarsis* is found from Wellington north to Auckland, and has been recorded on several offshore islands – Poor Knights Islands, Great Barrier Island and Middle Island in the Mercury group (Johns, 2001). Three long ovipositor species also occur in the NI, *H. maculifrons*, *H.* "elegans" and *H.* "otekauri", the latter two species are only found in northern NI.

1.3 Taxonomic history and systematics of Hemiandrus pallitarsis

The taxonomic history of *Hemiandrus pallitarsis* has been turbulent, due in part to poor species descriptions and confusion over early species names. Walker (1869), described two species: *Ceuthophilus* (?) *lanceolatus* from a male weta collected by H. Drew Esq. half a mile into a cave; and *Libanasa pallitarsis* from a male, the collection locality unknown. In 1896, Hutton synonymised *C.* (?) *lanceolatus* with a cave weta species, *Macropathus edwardsii,* as it was supposedly collected from the same location as three of Walker's other *Macropathus* species. However, the location and collection information of the *C.* (?) *lanceolatus* holotype was likely confused. Kirby (1906), re-examined Walker's type specimens and placed *C.* (?) *lanceolatus* into the genus *Onosandrus* Ståll, 1876, and also synonymised *Libanasa pallitarsis* under the new combination *Onosandrus* (?) *lanceolatus* Walker, 1869. Later Hutton (1899), synonymised *O.* (?) *lanceolatus* with *O. pallitarsis.* So at this stage in the taxonomy, both *C.* (?) *lanceolatus* and *L. pallitarsis* appear to be synonymous with *O. pallitarsis.* In 1938, Ander described a new genus and three new species in New Zealand; *Hemiandrus furcifer,* the type species for the genus which was collected from Palmerston North, *H. similis* collected in Wellington, and *H. bilobatus* also collected in Wellington. Following on from Ander, Salmon (1950) described nine new species and introduced a new genus, *Zealandosandrus* based on ovipositor length (fully developed

in *Zealandosandrus* and extremely short in *Hemiandrus*). In his revision of the New Zealand wetas, Salmon misidentified specimens and synonymised *O. pallitarsis* with *Z. maculifrons* on the basis of the shape of the subgenital plate. It was not until 1961, when Ramsay re-examined Walker's type specimen of *Ceuthophilus* (?) *lanceolatus* that he found it was actually an immature female (not a male), and possessed a very short ovipositor. He transferred *C.* (?) *lanceolatus* into the genus *Hemiandrus* Ander, 1938, and so it became *H. lanceolatus* (Walker, 1869). Ramsay observed that H. Drew Esq., the original collector of the holotype, did most of his collecting in the Wanganui area so *C.* (?) *lanceolatus* is possibly a conspecific of *H. furcifer* Ander, 1938. More recently, Johns (1997), placed all species into *Hemiandrus* based on initial phylogenetic research (Gerber, 1996) and a broader perspective on the systematics of the Anostostomatidae. Johns (1997), also synonymised *H. furcifer* with the new combination *H. pallitarsis*, and later suggested that *H. lanceolatus* may in fact be a doubtful name (Johns, 2001).

1.4 Mating behaviour of Hemiandrus pallitarsis

The reproductive behaviour of *Hemiandrus pallitarsis* involves complex signals, interesting reproductive structures, and paternal and maternal investment (Gwynne, 2002, 2004, 2005).

Vibratory drumming:

Although it has been suggested that pheromones may be involved in long distance communication, vibratory signals appear to be used for local mate attraction (Gwynne, 2005). Males drum their abdomens on the substrate, usually a plant leaf or stem, and females drum in apparent response to the male. Several other short ovipositor species also exhibit vibratory signalling – *H. bilobatus* (D.T., Gwynne, pers. comm. E.M., Chappell, pers. obs.), *H.* "promontorius", *H.* "onokis", and *H.* "vicinus" (Gwynne, 2005), and it has also been found in two long ovipositor species – *H. subantarcticus* (Butts, 1983), and *H. maculifrons* (Cary, 1981). Vibratory drumming in *H. pallitarsis* appears to be a pre-copulatory mechanism and does not occur after mating.

Copulation:

Copulation involves the production and transfer of a spermatophore from male to female. Like many Orthopterans, the spermatophore of *H. pallitarsis* consists of a sperm ampulla (containing the sperm) and a sperm-free spermatophylax (food gift) that the female feeds on while the sperm ampulla is expelled into her reproductive tract. However, unlike many Orthopterans, the two parts of the spermatophore are separate entities in *H. pallitarsis* (Gwynne, 2002). Males attach first to an accessory organ located on the ventral side of the female abdomen. This accessory organ is an elaborate elbowed structure, a modification of the sixth sternite. Whilst attached to this structure, the male then arches back and using the dorsal parts of his genitalia, attaches to the female's primary genitalia where he delivers the sperm ampulla. Once transferred, he then detaches from this area, remains attached to her accessory organ, and delivers the spermatophylax to her mid abdominal area (Gwynne, 2002, 2005). It has been suggested that this organ may be under sexual selection to acquire thesis nuptial gifts from males (Gwynne, 2005). After the male has separated completely from the female, she then promptly reaches under and grasps the food gift and proceeds to spend the next hour or so feeding upon it. During this time the male exhibits a mate guarding behaviour and remains close to the female, seemingly to prevent other males copulating with the her (Gwynne, 2005). *H. pallitarsis* also shows maternal care. Females lay their eggs into the bottom of their burrow and apparently stay with the eggs and nymphs for an extended period of time (Gwynne, 2005).

1.5 Thesis outline and chapter aims

The overall aim of this thesis is to examine intraspecific variation in *H. pallitarsis* from a broad perspective, encompassing morphological, genetic and behavioural characters. Chapter two sets out to document the morphological diversity across the entire range of *H. pallitarsis.* At present, there is some suggestion of regional morphological variation within this species (Johns, 2001), and a previous study found the size of the female accessory organ varied between geographically proximate populations (Gwynne, 2005). Morphometric variation across a landscape can be correlated with geographical differences in temperature (Atkinson, 1994), latitudinal or altitudinal differences (De Oliveira *et al.*, 2004), or as a result of sexual selection (Fairbairn, 1997). The suggested

morphological variation, and the potential for sexual selection on the female accessory organ in *H. pallitarsis*, suggests this is an interesting species in which to study morphological variation. This chapter specifically focuses on the morphometric variability, sexual size dimorphism and variation in the female accessory organ across a range of locations.

Chapter three focuses on the genetic variation and phylogeographic structure within *H. pallitarsis* populations, and how past geophysical events may have impacted current phylogeographic patterns. On the whole, there are few phylogeographical studies that have explicitly examined how patterns of genetic divergence within and among species correspond to Plio-Pleistocene geological and/or climatic changes in NI, New Zealand (Apte *et al.*, 2007; Morgan-Richards *et al.*, 2001; Stevens & Hogg, 2004). The majority of research has focused on the effects of mountain building and glaciation in the South Island, however important changes in the NI landscape have also occurred. For example, marine transgression in the lower NI during the Pliocene, habitat shifts during the glacial-interglacial cycles in the Pleistocene, and more recent volcanic activity in central NI. This chapter tested several hypotheses related to the geophysical history of the NI: 1) *H. pallitarsis* would have been restricted to the northern NI, then dispersed south as land emerged above sea level during the Plio-Pleistocene in what is now the lower NI; or 2) *H. pallitarsis* originated in the northern SI and range expansion has occurred in a south to north pattern following land uplift in the Pliocene; or 3) Pliocene impacts on the phylogeographic structure in *H. pallitarsis* has been masked by more recent climatic events and/or volcanic activity. These predictions were tested by analysing mitochondrial DNA cytochrome *c* oxidase subunit I (COI) sequence fragments from populations across the distributional range of *H. pallitarsis.*

Finally, the last chapter in this thesis investigated the variation in male vibratory signals between three populations of *H. pallitarsis* – Coromandel and two locations in Palmerston North, one north and one south of the Manawatu River. Geographical variation in sexual communication systems has been explained by environmental factors, such as temperature (Gerhardt & Huber, 2002; Toms *et al.*, 1993; von Helverson & von Helverson, 1994; Weissman, 2001), and social factors, such as sexual selection (Orci, 2007; Wilczynski & Ryan, 1999). The specific aim of this chapter was to test two hypotheses: 1) the two Palmerston North populations would exhibit more similar drumming behaviour patterns compared to the Coromandel population due to similarity in morphometrics and geographic proximity, or 2) drumming patterns on the

northern side of the Manawatu River would be more similar to those in Coromandel based on genetic similarities.

Collectively, these three chapters aim to give an insight into the degree of intraspecific variation that can be found in a single species. At the same time some interesting biological, behavioural and historical phylogeographic patterns are found.

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Chapter 2: Morphometric Variation

Dissected female *Hemiandrus pallitarsis* accessory organs showing morphological variation

Chapter 2: Morphometric variation in *Hemiandrus pallitarsis*

2.1 Introduction

When we look at an organism, the first thing we tend to notice is morphology, that is, colour, size and shape. Traditionally, species were described as 'kinds' or 'types' based on morphological differences, a concept commonly referred to as the morphological species concept in which "Population A … was determined to be a different species from population B … if it was deemed to be sufficiently different from it by morphological characters." (Mayr, 1996, p. 269). Additionally, Darwin's morphological species concept (1859) described species as "varieties" between which there are no or few morphological intermediates. Although in some cases, changes in morphology, such as body size, can lead to changes in performance, and can result in evolutionary novelties (Koehl, 1996). The morphological species concept can be misleading as individuals, populations and species are often morphologically variable, and yet often species classified under the biological species concept are morphologically identical (i.e. cryptic species). However, detailed knowledge of the degree of morphological diversity among populations is always an important step towards a clear understanding of the species status of distinct populations. Thus the collection and description of morphological variation will always be an important element in evolutionary, taxonomic and ecological studies.

Within insect species, variation in morphological characters can be correlated with a range of environmental factors such as latitude and altitude (Cushman *et al.*, 1993; Hawkins & Lawton, 1995; Krasnov *et al.*, 1996; Smith *et al.*, 2000). In a study investigating the corn leafhopper, *Dalbulus maidis*, across a range of latitudes (5 - 28° S) and altitudes (16 -1628 m a.s.l) in Brazil, De Oliveira *et al.* (2004) found individual leafhoppers were larger, heavier and had darker pigmentation at higher latitudes, and

were also heavier at higher altitudes. This pattern follows Bergmann's rule which predicts increased body size at higher latitudes (Bergmann, 1847), and has been found in other insect groups including European ants (Cushman *et al.*, 1993) and some species of butterflies (Hawkins & Lawton, 1995). Ectotherms, however, generally follow the converse to Bergmann's rule - smaller individuals towards the poles (Mousseau, 1997; Park, 1949; Partridge & Coyne, 1997; Van Voorhies, 1997). This pattern has been attributed to season length as opposed to temperature as shorter seasons at higher altitudes result in reduced growth and development time (Blanckenhorn & Demont, 2004).

Morphological characters are also affected by sexual selection, where selective processes can result in sexual dimorphism (Fairbairn, 1997). The most obvious example of sexual dimorphism in animals is where one sex is considerably larger than the other, termed sexual size dimorphism (SSD). Males tend to be larger than females in birds and mammals (Alexander *et al.*, 1979; Payne, 1984; Ralls, 1977), whereas among invertebrates, females tend to be larger (Andersen, 1994; Blackith, 1957; Hurlbutt, 1987; Vollrath & Parker, 1992). Interestingly, however, among invertebrates both male-biased and female-biased SSD can occur, sometimes within the same family or subfamily (Fairbairn, 1990; Tseng & Rowe, 1999).

This thesis focuses on a species of ground weta in North Island, New Zealand, *Hemiandrus pallitarsis* (Orthoptera: Anostostomatidae). Like many Orthopterans, male ground weta produce a spermatophore which is transferred to the female genitalia during mating (Gwynne, 2002). As described earlier, *H. pallitarsis* males deliver the sperm ampulla to the female's primary genitalia and deliver the food gift (spermatophylax) to her mid abdomen area, approximately where the accessory organ is located. It has been suggested that this accessory organ may be under sexual selection to acquire these nuptial gifts from males (Gwynne, 2005). Morphometric variation in this accessory organ has been studied in two *H. pallitarsis* populations approximately 40 km apart, Palmerston North and Kiriwhakapapa (Gwynne, 2005). The organ was found to vary by more than 50% of its total length (range 0.9 – 1.4 mm), and although differences between the two populations were not explored, the data suggested both populations have overlapping

ranges in accessory organ length (Palmerston North, 0.9 - 1.3 mm; Kiriwhakapapa, 1.1 - 1.4 mm).

As well as variation in the female accessory organ, several authors have alluded to other morphological variation within *H. pallitarsis.* For example, Johns (2001) noted that populations on the islands close to Northland and the Coromandel Peninsula have slight differences. In addition, *Hemiandrus* has been recorded on many islands east of the Coromandel Peninsula, all of which are either nature reserves, and/or wildlife sanctuaries, but doubt exists in the identification of ground weta on some of these islands. For example, *Hemiandrus similis* was recorded on Red Mercury Island in the Mercury group (Johannesson, 1972; Moeed & Meads, 1987). *H. similis* is synonomous with *H. bilobatus* (Salmon, 1950) and is otherwise only known from the Wellington region, Matiu/Somes Island, Brothers Island in the Cook Strait and Mana Island off the Kapiti coast. Therefore, it would be unexpected to find *H. bilobatus* on an island over 500 kilometres to the north. However, if the species of ground weta on Red Mercury Island is correctly identified as *H. bilobatus* (syn. *H. similis*), then this would present an interesting species disjunction similar to that found in Whitaker's skinks (Towns, 1985). Therefore, it is important to accurately establish the taxonomic status of ground weta on these islands, and the mainland, before undergoing any genetic investigations.

Given the great diversity of accessory organ size, and suggestions of regional morphological variation within *H. pallitarsis* (Johns 2001), the current study documents morphological diversity within the entire range of *H. pallitarsis* from latitude 36° to 41°. Specifically, patterns of morphometric variability, sexual size dimorphism, and variation in the female accessory organ are studied.

2.2 Methods

2.2.1 Sampling sites and collections

A total of 170 *Hemiandrus pallitarsis* individuals were collected between November 2006 and April 2007 from 15 locations in North Island (NI), New Zealand (Fig. 2.1). Specimens from two mainland locations (Wellington and Palmerston North) were available in the collection of Steve Trewick and Mary Morgan-Richards at the Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Palmerston North. Specimens from Great Barrier Island were provided by John Early at the Auckland Museum. In total, eleven mainland sites and four islands were sampled across NI.

Weta were hand collected by searching the ground, low shrubs and tree trunks after dark with a head lamp or torch light, and were found in a variety of habitats ranging from urban gardens to native forest. At most locations, sufficient numbers of weta could be collected within 2 - 3 hours; some areas where ground weta were abundant over 20 adults could be found within 20 minutes.

Specimens were initially frozen at -20°C and were later preserved in 95% ethanol. Individuals were identified to species level using the following morphological characters: one foretibial spine, three prolateral spines and four retrolateral spines on the mesotibia, 70% cover of fine pilosity on the fourth maxillary palps and bare third maxillary palps (Johns, 2001). Weta were deemed to be adult based on descriptions by Johns (2001) - in the penultimate instar in males, the falci (hook-like structures) on the last tergite are simple rounded knobs, in adults the falci become blackened hooks, whereas adult females have reduced or absent apical styles on the ovipositor but were also judged on development of the sixth abdominal sternite (accessory organ).

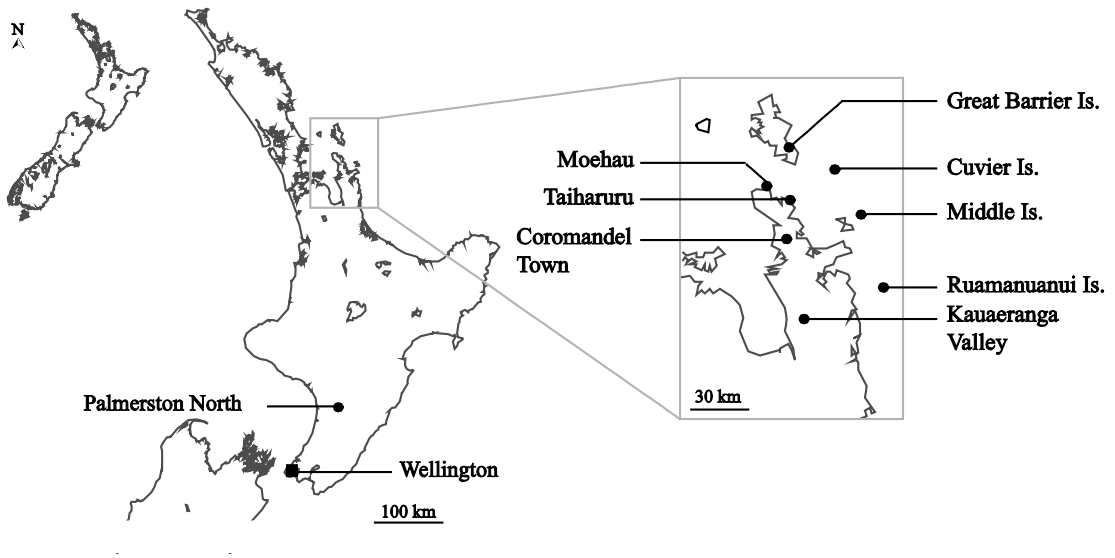


Figure 2.1. Collection locations of *Hemiandrus pallitarsis* in North Island, New Zealand. Inset shows collection locations on the Coromandel Peninsula.

2.2.2 Body measurements

91 adult weta (51 males and 40 females) were initially measured for fourteen morphological traits (Fig. 2.2a-h). Morphological structures were measured with digital callipers (AIA) accurate to 0.01mm. Each female accessory organ was dissected and fixed onto a slide with mounting fluid and then measured using a stereomicroscope (Nikon Alphaphot YS) fitted with a calibrated eyepiece. A correlation matrix for nine of the fourteen morphological traits (male and female reproductive characters were not used in the correlation analysis), reduced the number of measurable non-reproductive traits to six, with the length of the metafemur used as an indicator of the length of all other appendages (Appendix A). In fact, the metafemur length was highly correlated (i.e. 90%) to all other leg measures (protibia, mesotibia and metatibia length). Despite the high correlation between the width and length of the head (93.8%), both measurements were included in further analyses to determine whether there was any shape relationships between the head and body measurements.

Subsequently, eleven morphological traits were measured in 170 adult weta (97 males and 73 females) from ten populations. Measurements for males included the pronotum length (Pr), metafemur length (Mf), fastigium width (Fa) - distance between

lateral ocelli -, head width (Hw) and length (Hl), distance from fastigium to hind coxa (used as a descriptor of body length (BL), due to distortions of the abdomen associated with alcohol fixation), length of the subgenital plate (Sg), maximum (MxSg) and minimum (MnSg) width of the subgenital plate. The first eight of these measurements were also taken for females, as was the mean length of the left and right tines in the fork of the accessory organ (LAO), and width of the accessory organ at the base of the fork (WAO).

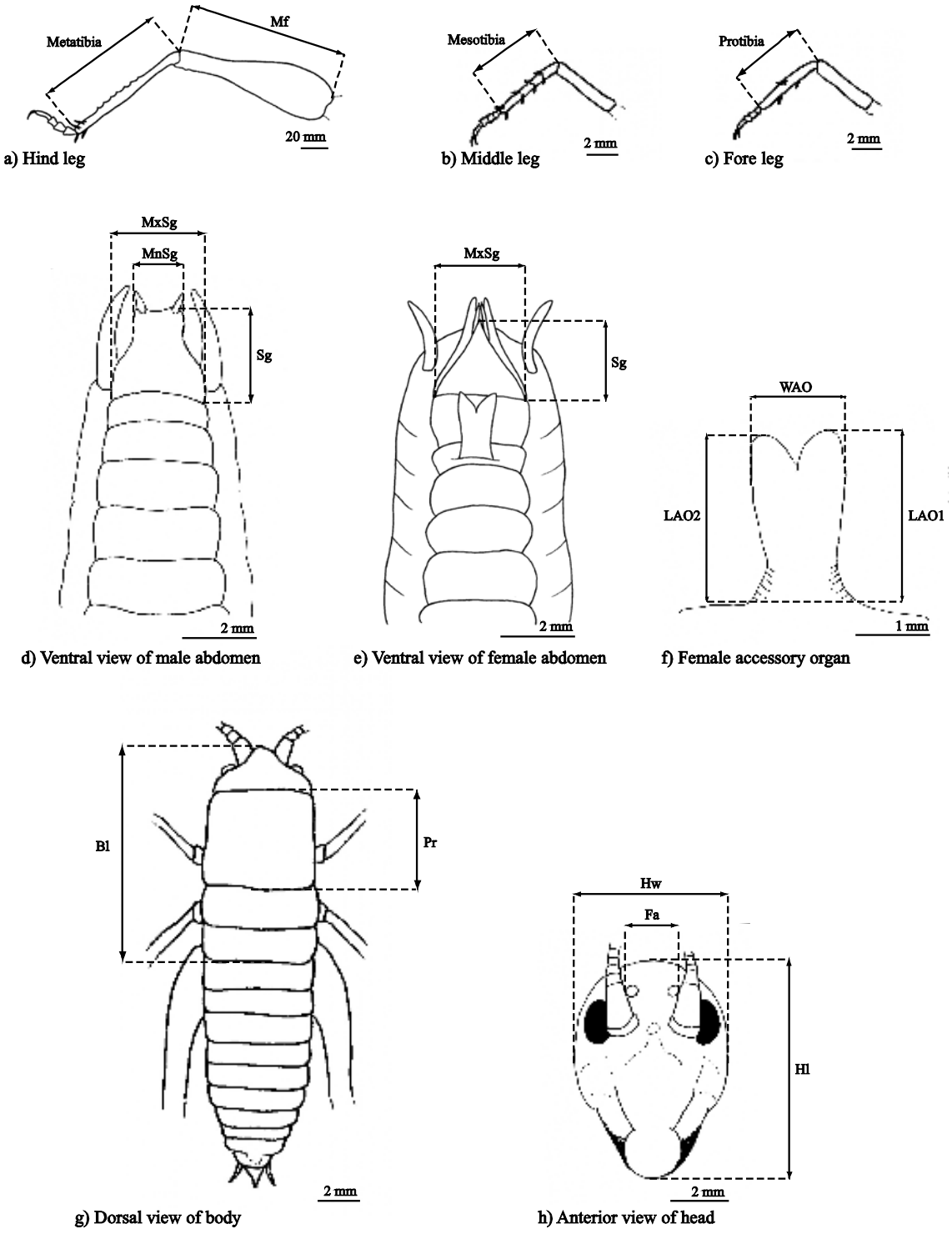


Figure 2.2a-h. Morphometric characters measured for *Hemiandrus pallitarsis* specimens. Abbreviations as follows: metafemur (Mf), length subgenital plate (Sg), maximum width subgenital plate (MxSg), minimum width subgenital plate (MnSg), length of right (LAO1) and left (LAO2) tine of the accessory organ fork, width of accessory organ (WAO), head length (Hl), head width (Hw), fastigium width (Fa), distance between third tergite and anterior fastigium (Bl), length pronotum (Pr).

2.2.3 Measurement error

Of the 169 weta that were measured, fifteen were selected at random (via a random number generator) and were re-measured for all eleven traits without reference to the original measurements. For each of the eleven traits, the first measurement taken was subtracted from the second measurement and the mean of the difference was calculated for each trait. A t-test comparison was employed using the statistical software SPSS version 4.0 (SPSS, Chicago, Ill., USA), to test the hypothesis that the mean was equal to zero. If the mean of the difference between the first and second measure was not equal to zero, then that trait was dropped from subsequent analyses.

2.2.4 Statistical analyses

MINITAB v. 14.0 software was used to generate standard descriptive statistics and perform multivariate analysis of the morphometric data. A paired *t*-test was used to confirm the observation that females are larger than males. The data were log-transformed to improve normality as several of the traits did not follow a normal distribution (Anderson-Darling Normality Test; $p > 0.10$). Variability of morphological characters among populations was analysed using both univariate and multivariate methods. Univariate comparisons were performed by one-way analysis of variance (ANOVA). If significant differences among the populations were found ($p < 0.05$), then Tukey's posthoc test was used to determine which populations did not differ from each other and which populations were significantly different from all other populations.

Multivariate analysis included principle component analysis (PCA); an ordination technique which is a form of data reduction that still maintains the maximum information of the dataset. PCA summarises multivariate information (in this case: eleven body measurements, nine locations, and two sexes) and allows the detection of similarities and differences in the data. These patterns are then represented graphically by plotting the first principle component against the second principle component. Points on the graph which are close together are more similar morphometrically than those which are further apart. Principle components can be used to give descriptions of the general morphology of the organism (e.g. body size, body shape). Post hoc tests and PCA were not performed for the length or width of the accessory organ (LAO and

WAO, respectively) as the accessory organ could not be dissected out of the museum specimens (i.e. Great Barrier Island individuals).

Discriminant function analysis (DFA) was performed to determine which variables best predict the population origin of ground weta. DFA calculates the percentage of each population which is correctly assigned to their population of origin based on the morphometric measurements. Cross-validation was employed whereby one case is omitted at a time, the remaining data are used to recalculate the classification function, and then the omitted case is classified.

Variation in body size with latitude was determined by plotting each population mean for each morphometric character against latitude, which ranged from 36° ′18 (Great Barrier Island) to 41° 16(Wellington). Pearson *r* correlation coefficients were used to evaluate the relationship between body size and latitude.

2.3 Results

2.3.1 Measurement error

For ten of the eleven morphometric measurements, the mean was not significantly different from zero (Appendix B). Therefore all traits except the minimum width of the male subgenital plate were used in further morphometric analyses.

2.3.2 Sexual size dimorphism

Analysis of all six traits measured for both males and females confirmed the observation that females are significantly larger in all body measurements than their male counterparts (*t*-test, $p < 0.001$, Table 2.1). Because of the significant sexual dimorphism, males and females were treated separately in further analyses.

Table 2.1. Means and standard deviations (SD) for six morphometric traits of adult male and female *H. pallitarsis*.

	Mean (mm) ±SD		Paired *t*-test
	Males	Females	*p*
	(*n* = 97)	(*n* = 73)	
Pronotum (Pr)	4.82±0.37	5.29±0.40	< 0.001
Metafemur (Mf)	12.66±0.84	13.48±0.91	< 0.001
Fastigium (Fa)	1.40±0.11	1.60±0.12	< 0.001
Body length (BL)	10.96±1.01	11.90±0.77	< 0.001
Head width (Hw)	4.91±0.32	5.53±0.34	< 0.001
Head length (Hl)	7.36±0.44	8.23±0.49	< 0.001

2.3.3 Morphometric variation among populations

One-way ANOVA revealed that all morphometric variables differed significantly among the ten populations of male weta (Table 2.2), and between the nine populations of female weta (only one adult female was measured from Wellington and so was not used in morphometric analyses) (Table 2.3). Tukey's posthoc test showed that for six of the eight morphometric variables, male weta from Palmerston North were significantly smaller than male weta from all other populations and that the eight Coromandel Peninsula populations and Wellington are relatively poorly differentiated from each other. In contrast to males, post hoc tests showed that all female weta populations are relatively poorly differentiated from each other.

For male weta, principal component analysis showed that the first and second principle components, PC1 and PC2, explained 68.3% and 11.2% of the total variance, respectively (Fig. 2.3). All morphometric characters had positive loadings on PC1, in which the highest loadings were associated with the head size and the length of the pronotum (Appendix C). The highest loadings on PC2 were the length of the subgenital plate and the width of the fastigium. Head shape, body length and the width of the fastigium had negative loadings on PC2. Scores from PC1 were used as general descriptors of body size, while scores from PC2 did not show a distinct relationship between measurements. PCA also supports the separation of the Palmerston North population. Adult male weta from Palmerston North are smaller in overall body size than weta from all other populations (Fig. 2.3).

For the female weta, all variables loaded positively on PC1; head length and head width yielded the highest loadings, followed by the length of the pronotum (Appendix D). Once again, scores from PC1 were used as general descriptors of body size. The length and maximum width of the subgenital plate had the highest loadings on PC2; all other scores on PC2 had negative loadings. Therefore, PC2 describes the size of the subgenital plate in relation to all other body parts. PC1 and PC2 accounted for 78.2% of the total morphometric variability. Figure 2.4 shows several outliers from Great Barrier Island, and a single outlier from Kauaeranga Valley. These individuals have smaller subgenital plates than all other weta. Overall, the majority of adult female weta from Palmerston North were smaller in body size than weta from the Coromandel

populations, although, unlike males, there is overlap in body size and shape with other populations (Fig. 2.4).

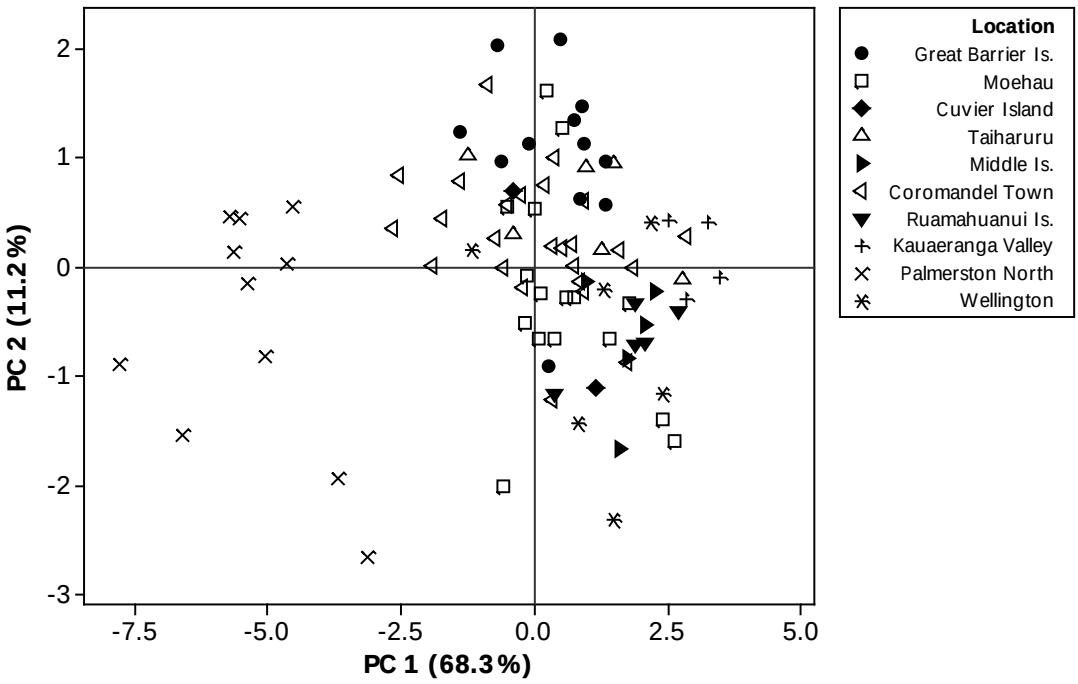


Figure 2.3. Principal component analysis on eight morphological traits of adult male *H. pallitarsis*. Percent of total variation marked on each axis.

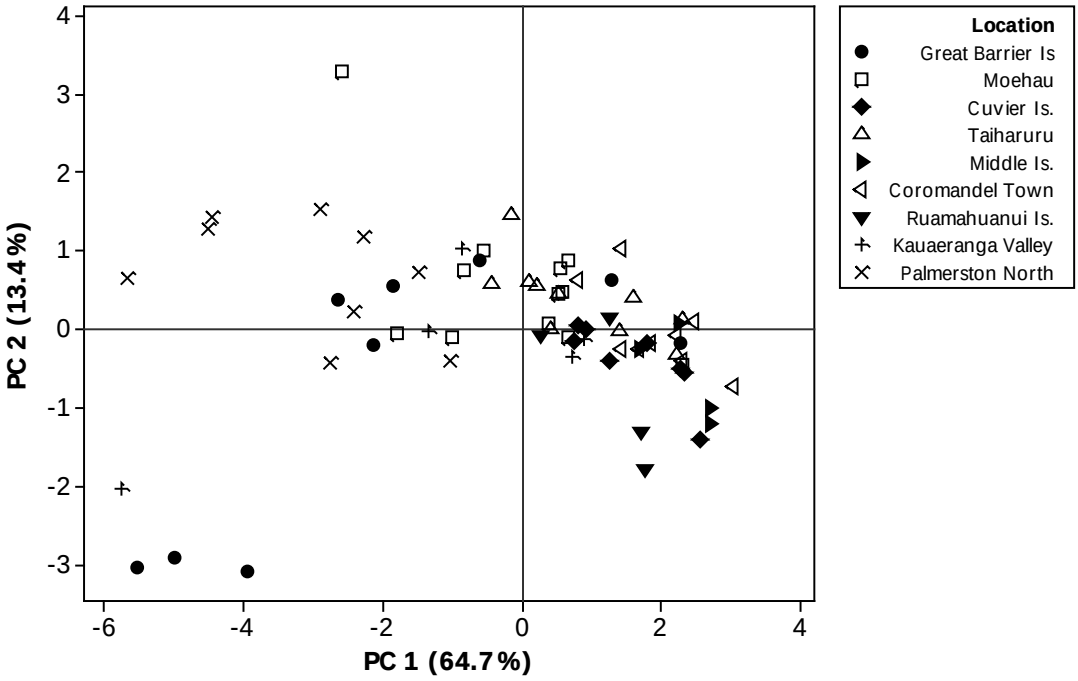


Figure 2.4. Principal component analysis on eight morphological traits of adult female *H. pallitarsis*. Percent of total variation marked on each axis.

Table 2.2. Measurements of adult male *H. pallitarsis* from ten populations before log transformation. All measurements are in millimetres (mm). The statistical significance of differences among populations has been assessed using one-way ANOVA.

	Pronotum (Pr)	**Metafemur (Mf)**	**Fastigium (Fa)**	**Body length (BL)**	**Head width (Hw)**	**Head length (Hl)**	**Subgenital plate (Sg)**	**Width Subgenital plate (MxSg)**
	Mean ± SD							
Great Barrier Island	5.03±0.27	12.90±0.37	1.37±0.07	11.09±0.69	4.72±0.15	7.33±0.23	2.86±0.19	3.11±0.10
Cuvier Island	5.23±0.29	12.64±0.49	1.46±0.09	11.14±0.46	5.02±0.17	7.47±0.30	2.78±0.15	2.85±0.17
Moehau	5.60±0.20	12.48±0.61	1.43±0.03	11.66±0.45	4.87±0.38	7.49±0.32	2.80±0.32	2.72±0.16
Middle Island	5.40±0.21	13.20±0.60	1.37±0.09	11.27±0.69	5.05±0.22	7.44±0.32	2.83±0.08	2.90±0.18
Coromandel Town	5.74±0.16	12.57±0.23	1.48±0.04	12.31±0.80	5.27±0.18	7.67±0.24	2.69±0.09	3.15±0.12
Taiharuru	5.61±0.24	13.08±0.43	1.35±0.10	10.73±0.76	4.92±0.18	7.35±0.28	2.72±0.12	2.77±0.23
Ruamahuanui Island	5.64±0.22	12.45±0.21	1.49±0.05	11.77±0.65	5.29±0.15	7.78±0.25	2.80±0.09	2.91±0.16
Kauaeranga Valley	5.07±0.37	13.82±0.58	1.42±0.09	11.71±0.81	5.18±0.28	7.87±0.37	2.85±0.22	3.01±0.11
Palmerston North	4.68±0.22	11.06±0.89	1.31±0.11	9.43±1.05	4.41±0.39	6.70±0.66	2.44±0.14	2.39±0.21
Wellington	5.06±0.23	12.79±0.41	1.56±0.09	11.26±0.79	5.03±0.25	7.55±0.35	2.74±0.18	2.98±0.16
ANOVA *p*	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 2.3. Measurements of adult female *H. pallitarsis* from nine populations before log transformation. All measurements are in millimetres (mm). The statistical significance of differences among populations has been assessed using one-way ANOVA.

	Pronotum (Pr)	Metafemur (Mf)	Fastigium (Fa)	Body length (BL)	Head width (Hw)	Head length (Hl)	Subgenital plate (Sg)	Width Subgenital plate (MxSg)	Length Accessory Organ (LAO)	Width Accessory Organ (WAO)
	Mean ± SD									
Great Barrier Island	5.03±0.27	13.12±0.99	1.46±0.11	12.23±0.91	5.15±0.26	7.81±0.44	1.77±0.56	2.25±0.47	-	-
Cuvier Island	5.23±0.29	13.42±0.60	1.61±0.07	11.51±0.30	5.49±0.29	8.21±0.46	2.12±0.29	2.55±0.13	2.49±0.21	1.28±0.13
Moehau	5.60±0.20	13.90±0.30	1.78±0.06	12.11±0.83	5.76±0.08	8.57±0.22	2.03±0.20	2.52±0.14	2.22±0.15	1.27±0.16
Middle Island	5.40±0.21	13.91±0.37	1.59±0.06	12.22±0.46	5.57±0.22	8.35±0.24	2.28±0.12	2.55±0.14	2.70±0.20	1.37±0.20
Coromandel Town	5.74±0.16	13.88±0.32	1.66±0.03	12.90±0.68	6.00±0.13	8.86±0.27	2.08±0.04	2.62±0.19	2.61±0.12	1.42±0.16
Taiharuru	5.61±0.24	14.50±0.47	1.62±0.09	12.08±0.59	5.76±0.20	8.51±0.27	2.02±0.29	2.53±0.40	2.67±0.46	1.28±0.20
Ruamahuanui Island	5.64±0.22	13.50±0.59	1.67±0.10	12.31±0.53	5.79±0.14	8.66±0.22	1.95±0.11	2.45±3.7	2.33±0.10	1.39±0.18
Kauaeranga Valley	5.07±0.37	13.04±0.98	1.51±0.09	11.70±0.69	5.44±0.34	8.08±0.40	1.92±0.41	2.29±0.45	2.58±0.22	1.24±0.14

Palmerston North	4.68±0.22	12.11±0.54	1.52±0.09	10.93±0.56	5.16±0.27	7.61±0.44	1.87±0.21	2.32±0.19	2.19±0.18	1.13±0.25
ANOVA *p*	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.015	0.011	-	-

2.3.4 Population classification

The Discriminant Function Analysis (DFA) on 97 adult males using the ten collection localities resulted in 63.3% correct classification using cross-validation (Table 2.4). All individuals from Palmerston North were correctly assigned to their population of origin and Great Barrier Island, Ruamahuanui Island, and Kauaeranga Valley populations only had one individual misclassified in each location. The Discriminant Function Analysis on 72 adult females correctly classified 42.9% of adult female weta to nine populations (Table 2.5). As for the males, Palmerston North had the highest percentage of correctly classified individuals (55.6%). In general, however, females were not as well classified into their populations of origin as the males.

Table 2.4. Percentage correct *a posteriori* classification of populations based on the eight morphometric variables for male *H. pallitarsis.*

Classified as	True group									
	1	2	3	4	5	6	7	8	9	10
1 Great Barrier Island	11	1	1	0	0	0	0	0	0	0
2 Moehau	0	6	1	1	0	1	0	0	0	2
3 Cuvier Island	1	2	0	0	0	2	0	0	0	1
4 Taiharuru	0	1	0	2	0	2	0	0	0	0
5 Middle Island	0	0	0	0	3	0	1	0	0	0
6 Coromandel Town	0	1	0	2	0	19	0	1	0	0
7 Ruamahuanui Island	0	1	0	0	2	0	4	0	0	1
8 Kauaeranga Valley	0	0	0	1	0	0	0	3	0	0
9 Palmerston North	0	0	0	0	0	0	0	0	11	0
10 Wellington	0	4	0	0	0	1	0	0	0	2
% Correct	**91.7**	**37.5**	**0**	**33.3**	**60**	**76**	**80**	**75**	**100**	**33.3**

Table 2.5. Percentage correct *a posteriori* classification of populations based on the eight morphometric variables for female *H. pallitarsis.*

Classified as	True group								
	1	2	3	4	5	6	7	8	9
1 Great Barrier Island	3	0	0	0	0	0	0	0	0
2 Moehau	0	6	1	0	0	1	0	1	0
3 Cuvier Island	0	1	4	0	0	1	1	0	0
4 Taiharuru	5	2	1	5	0	3	0	1	0
5 Middle Island	0	0	0	1	1	0	1	0	0
6 Coromandel Town	0	0	0	3	1	4	1	1	0
7 Ruamahuanui Island	0	0	2	1	2	0	1	0	1
8 Kauaeranga Valley	1	1	0	0	0	0	0	1	3
9 Palmerston North	0	2	0	0	0	0	0	1	5
% correct	**33.3**	**50.0**	**50.0**	**50.0**	**25.2**	**44.4**	**25.0**	**20.0**	**55.6**

2.3.5 Variation in body size with latitude

There was no significant correlation between any of the eight morphometric characters measured and latitude for male weta when the Wellington population was included (Table 2.6). Re-running the analysis without the Wellington population results in significant negative correlations between six of the eight characters with latitude; pronotum length, metafemur length, body length, head length, subgenital plate length and width of the subgenital plate decreased with increasing latitude (Table 2.6; Fig 2.5). Four out of the ten measurements for females are significantly negatively correlated

with latitude; pronotum length, metafemur length, body length and width of the accessory organ were all smaller at higher latitudes (i.e. further south) (Fig. 2.5).

Table 2.6. Correlation coefficients (linear regression) between morphometric characters and latitude (Lat) in males (including and excluding the Wellington population) and females of *H. pallitarsis*.

Character	Males (incl. Wellington)			Males (excl. Wellington)			Females		
	N	r^2	*p*	*N*	r^2	*p*	*N*	r^2	*p*
Pr/Lat	10	0.2510	0.140 ns	9	0.6823	0.006 *	9	0.4449	0.050 *
Mf/Lat	10	0.2144	0.178 ns	9	0.5491	0.022 *	9	0.5640	0.020 *
Fa/Lat	10	0.0313	0.625 ns	9	0.3025	0.125 ns	9	0.1056	0.394 ns
Bl/Lat	10	0.2714	0.122 ns	9	0.6448	0.009 *	9	0.5213	0.028 *
Hw/Lat	10	0.1452	0.277 ns	9	0.4225	0.058 ns	9	0.2218	0.201 ns
Hl/Lat	10	0.1875	0.212 ns	9	0.5730	0.018 *	9	0.3411	0.099 ns
Sg/Lat	10	0.3697	0.062 ns	9	0.7797	0.002 *	9	0.1050	0.394 ns
MxSg/Lat	10	0.1414	0.285 ns	9	0.5761	0.018 *	9	0.1544	0.295 ns
WAO/Lat	-	-	-	-	-	-	8	0.5055	0.048 *
LAO/Lat	-	-	-	-	-	-	8	0.2830	0.174 ns

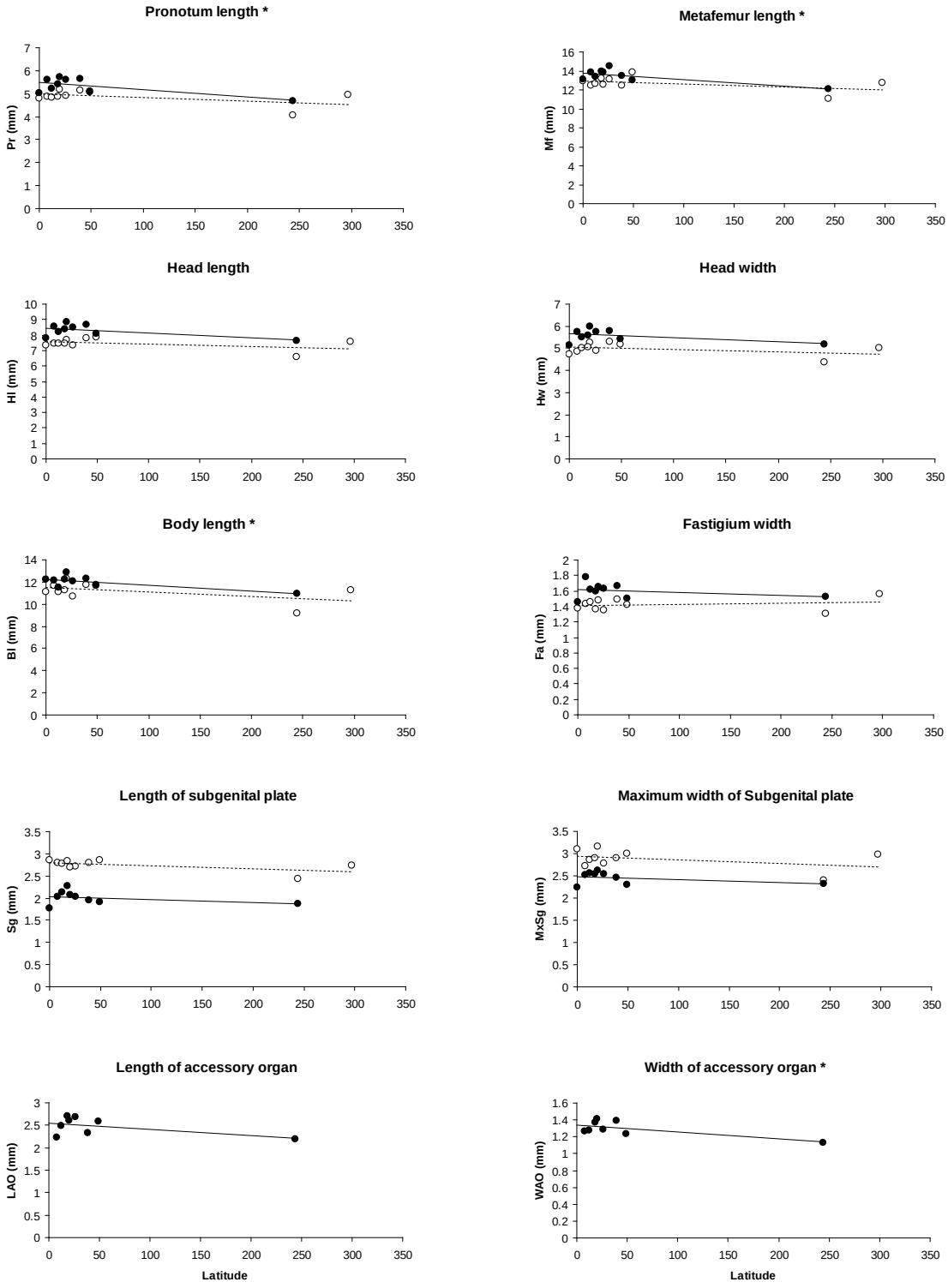
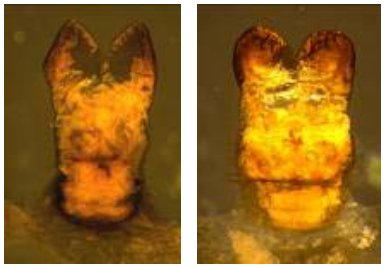


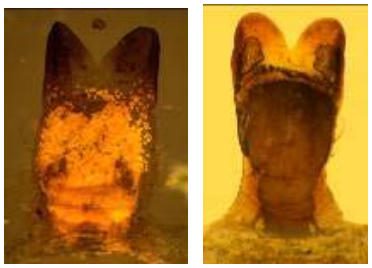
Figure 2.5. Relationships between the means of morphometric characters and latitude in populations of *H. pallitarsis* from North Island, New Zealand. Females, ●; males, ○. * indicates characters significantly correlated with latitude in female populations (Pearson r; $p < 0.05$). All regressions are linear. Latitude is expressed in minutes from Great Barrier Island locality (0′) as this is the northern most location.

2.3.6 Variation in accessory organ shape

Cuvier Island

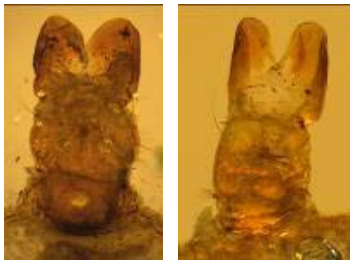


Middle Island



Taiharuru

Coromandel Town



Palmerston North

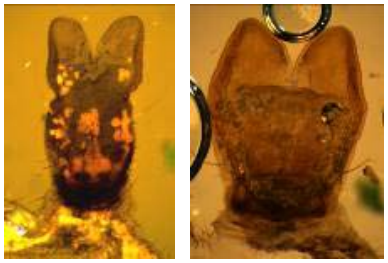


Figure 2.6. Images of dissected female accessory organs showing variability in shape from Cuvier Island, Middle Island, Taiharuru, Coromandel Town and Palmerston North.

Table 2.7. Pearson correlation *r* of length of the female accessory organ (LAO) with length of pronotum, metafemur and body length.

	Pronotum	Metafemur	Body length
LAO			
Pearson correlation *r*	0.384	0.518	0.374
p value	0.002	0.000	0.004

2.4 Discussion

Hemiandrus pallitarsis was identified from several new locations within the distributional range described by Johns (2001). This study was able to identify *H. pallitarsis* from Cuvier Island and one of the Alderman Islands, previously only identified to the genus level. Significant sexual size dimorphism, and population morphometric variation was found in this study. However, the level of morphological variation found provides no indication of multiple 'morpho-species' at different populations in North Island, New Zealand, but is consistent with levels of intraspecific variation.

2.4.1 Sexual size dimorphism

There was marked sexual dimorphism in *H. pallitarsis.* In all morphometric characters, females were significantly larger than males. This trend of female-biased sexual size dimorphism (SSD) is common throughout insects (Teder & Tammaru, 2005) and is also found within many Orthopteran families (e.g. Acridoidea, Bidau & Marti, 2007; Tettigoniidae, Gwynne, 1984; Podisminae, Tatsuta & Akimoto, 1998). Large body size in females is positively correlated with female fecundity (number of eggs) in a wide range of insects (Blanckenhorn, 2000; Fairbairn, 1997; Honek, 1993). Therefore, a linear increase in fecundity with body size may provide evidence for sexual selection where males prefer larger, more fecund mates (Andersen, 1994; Svensson & Petterson, 1987). On the other hand, Issac (2005), who studied mammals not insects, suggested decreased male-male competition could result in female-biased SSD, and that males are actually smaller rather than the females being larger. In *H. pallitarsis*, however, there is some evidence for sexual selection on some female traits. Gwynne (2005), experimentally removed the accessory organ from nine females, and found that in all cases copulation was prevented. Additionally, in observations of a mating pair in the field, a male was seen to abdominally probe a females mid-abdomen (approximately where her accessory organ is located), then after several minutes he rejected her and moved away (Gwynne, 2005). So it appears that males may be choosing mates based on this female accessory organ. It has also been suggested that the length of this female

accessory organ may be positively correlated with fecundity (number of eggs in a brood), and therefore, may be under sexual selection to acquire spermatophylaxes from males (Gwynne, 2005). Although Gwynne did not find a correlation between accessory organ length and body size (based on length of the pronotum), a positive correlation was found in this study with three measures of body size (length of the pronotum, length of the metafemur, and an approximation of body length; $p < 0.005$) (Table 2.7). As a result, the length of the accessory organ does appear to increase linearly with body size. Interestingly though, the length of the accessory organ was not correlated with latitude, but the pronotum, metafemur and body length characters were.

Due to the lack of general biological knowledge of *H. pallitarsis* it is unclear how the difference in size between males and females is achieved. Three mechanisms have been hypothesized to lead to sexual size dimorphism: 1) one sex is larger at birth/hatching; 2) one sex grows at a faster rate; or 3) one sex grows for a longer time period, in insects for example, the larger sex may have additional instars (Esperk & Tammaru, 2006). Therefore, future research examining the growth patterns and basic biology of ground weta will help to elucidate the mechanism leading to sexual size dimorphism in *H. pallitarsis.*

2.4.2 Geographical morphometric variation

Overall male body size appears to separate Palmerston North from the Coromandel populations and Wellington, but there is no significant separation in female body size between these populations. Male weta are significantly smaller in Palmerston North, a trend also seen in female weta. Female body size appears to be significantly correlated with latitude - smaller weta occur at higher latitudes. Male body size does not appear to be correlated with latitude until Wellington is removed from the data set. Overall, *H. pallitarsis* does not follow Bergmann's rule like previous insect examples (Cushman *et al.*, 1993; De Oliveira *et al.*, 2004; Hawkins & Lawton, 1995), but there is evidence that females follow the converse to Bergmann's rule, (Atkinson, 1994; Masaki, 1978; Mousseau & Roff, 1989; Mousseau, 1997; Partridge & Coyne, 1997; Telfer & Hassall, 1999). It is important however, to point out that the pattern of decreasing body size with increasing latitude is likely influenced by sampling error. Female weta from the

Wellington area were not measured, if they had been, we may have seen a similar pattern to what was shown in the male weta – no geographical cline in body size.

The observed difference in weta body size in the Palmerston North population may relate to environmental conditions, such as season length (Blanckenhorn & Demont, 2004). Shorter seasons at high latitudes restrict the length of time for foraging, growth and development, and insects end up smaller in overall size (Blanckenhorn & Demont, 2004). Previous research on ground weta (from the Christchurch area) suggests they over-winter at approximately the sixth and seventh instars (Wahid, 1978), and growth and development mostly occurs during the warmer months (November – April). Therefore, if Palmerston North has a considerably shorter summer season, there would be less development time available, resulting in smaller weta.

The lack of correlation between body size and latitude, but the obvious difference in body size of individuals from the Palmerston North population, suggests sexual selection, rather than natural selection, is acting on body size. Evidence for this may be found through examining an aspect of the mating behaviour of *H. pallitarsis.* Like many insects, males provide a nuptial meal to the female as a form of paternal investment (Thornhill & Alcock, 1983). In some cases larger males transfer larger nuptial gifts to females (Eisner & Meinwald, 1995; Fedorka & Mousseau, 2002b). Moreover, Fedorka and Mousseau (2002a) found that "…direct fecundity selection for larger gifts placed indirect positive selection on male body size due to the body size/gift correlation…" (p. 594). Consequently, it is possible that sexual selection is indirectly acting on male body size in *H. pallitarsis* as a result of female preference for larger nuptial gifts. The fact that Palmerston North males are smaller may be due to population density and competition. For example, Gwynne (1984) studied a species of Mormon cricket in populations of varying densities, and found in high density populations males selected larger females. Gwynne suggested this may be due to food limitation. Of course, it is difficult to imagine that all the populations sampled in the present study except for Palmerston North are at high densities and food limited given the suite of animals which predate upon them (Johns, 2001).

In conclusion, this study was able to show significant sexual size dimorphism in *H. pallitarsis*, but further study is needed to understand how these size differences are ontogenetically achieved. Additional research is also required to fully understand the level of morphological variation within *H. pallitarsis*, through increased sampling of populations between the Coromandel Peninsula and Wellington regions, and also across

North Island, from the east coast to the west coast. This information, in conjunction with information on environmental effects such as temperature and season length, may provide a clearer picture of whether *H. pallitaris* morphometrically varies clinally across the landscape.

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Chapter 3: Phylogeography

Male *Hemiandrus. pallitarsis* ready to jump from a branch in a Coromandel forest.

Chapter 3: Phylogeography of a ground weta, *Hemiandrus pallitarsis*

3.1 Introduction

Over the past five million years the New Zealand landscape has undergone mountain building in the South Island (SI), land uplift in the North Island (NI), and periodic expansion and contraction of glaciers and vegetation zones due to climatic changes. Research into how these past geological and climatic events might have affected the present-day geographic structure of populations has been comprehensively studied in recent years. For example, in the last ten years over 30 phylogeographic studies of New Zealand taxa have been published. The majority of these studies have focused on phylogeographic patterns in the SI, due its dynamic geological history, and implicate Pliocene mountain-building as drivers of the distribution and structure of many plant taxa (Heenan *et al.*, 2002; Lockhart *et al.*, 2001; Winkworth *et al.*, 2002), and invertebrate taxa (Buckley & Simon, 2007; Chinn & Gemmell, 2004; Trewick *et al.*, 2000; Trewick & Morgan-Richards, 2005). In addition to the uplift of the Southern Alps, Pleistocene glaciation and climate change appear to have influenced contemporary phylogeographic patterns in many SI taxa (e.g. moa, Baker *et al.*, 2005; cicadas, Buckley & Simon, 2007; snails, Neiman & Lively, 2004; weta, Trewick, 2001). Although considerable research has been devoted to understanding how Plio-Pleistocene geological and climatic events may have influenced distribution patterns among many SI organisms, rather less attention has been paid to how these past events may have impacted NI organisms.

Of the 30 plus papers that have been published in the last 10 years on phylogeography in New Zealand, approximately half included some representation of NI taxa (e.g. greenshell mussel, Apte & Gardner, 2002; sea-star, Ayers & Waters, 2005; cicada, Buckley *et al.*, 2001; freshwater fish, Burridge *et al.*, 2006; kokako, Murphy *et al.*,

2006; blue duck, Robertson *et al.*, 2007). However, several studies failed to sample across the entire distributional range of a species (Smith *et al.*, 2006), or instead focused on species restricted to the northern half of the NI (e.g. Spencer *et al.*, 2006; Hare *et al.*, 2008; Gleason *et al.*, 1999). Few studies have explicitly examined how patterns of genetic divergence within and among species correspond to Plio-Pleistocene geological and/or climatic changes in the NI (Apte *et al.*, 2007; Morgan-Richards *et al.*, 2001; Stevens & Hogg, 2004). Those that have analysed intraspecific genetic variation across NI have found apparently incongruent results. For example, Morgan-Richards *et al.*, (2001) used mitochondrial DNA sequence data to investigate the date and pattern of divergence within the Auckland tree weta (*Hemideina thoracica*), and to determine if the eight distinct chromosomal races were fixed by Pleistocene or Pliocene isolation events. They found mtDNA lineages and chromosomal races were closely correlated to Pliocene islands in Northland, and that *H. thoracica* probably expanded its range south into the lower NI after volcanic activity in the central NI and climate fluctuations during the Pleistocene. In contrast to this 'north to south' pattern, Apte *et al.*, (2007) found strong evidence for mid to late Pliocene 'south to north' movement in freshwater crayfishes (koura) (*Paranephrops planifrons*). Results from the cytochrome oxidase I (COI) gene revealed several patterns: firstly, koura appear to have dispersed from northern SI and Wairarapa into the east and west coasts of NI, from there they moved into northern NI, and then south again back to Wairarapa. This south to north pattern of dispersal in koura contradicts the author's prediction that *P. planifrons* survived in the northern archipelago during the Pliocene marine transgression, and subsequently dispersed south as the land uplifted. Given the current knowledge of the geological and climatic history of NI, both these patterns of lineage evolution could be predicted. Alternatively, it is also possible that the phylogeographic patterns observed have been masked by more recent events such as volcanism or habitat changes. Therefore, a detailed knowledge of the geophysical history of the NI is useful to establish the most likely explanation for contemporary phylogeographic patterns in NI taxa.

3.1.1 North Island Plio-Pleistocene geophysical history

Several authors have produced palaeogeographic maps (with varying degrees of similarity) showing the approximate maximum extent of marine transgression in the lower NI at the early Pliocene – an area known as the 'proto-strait' (Fleming, 1979; Kamp, 1982; Kamp & Vucetich, 1982; Lewis & Carter, 1994; Stevens, 1974; Suggate *et al.*, 1978). In general, all authors agree that land existed in what is now the upper half of NI with the southern coastline expanding from north of Taranaki (between Waitara and the Mokau River), east across the central NI to approximately 40 km from the east coast, and then up to East Cape. Rogers (1989) however, highlights some of the variation between authors in the areas suggested to be covered by the Pliocene marine transgression, such as the Kaimanawa Mountains and the Kaweka Ranges (Fleming, 1979; Grindley, 1960; Kamp, 1982; Suggate *et al.*, 1978). Additionally, it is agreed that there was a Pliocene land connection between Wairarapa in NI, and the Marlborough Sounds in SI (see Rogers, 1989), but the estimated position and size of that land connection is unclear. For example, Suggate *et al*., (1978) and Lewis and Carter (1994) (and references therein) suggest during the Opoitian (lower Pliocene, 5.28 - 3.6 Ma) there was land from the Kapiti Coast to just south of Wanganui - termed the 'Wanganui-Marlborough Shield' (Fig. 3.1A).

Stevens (1974), puts forward a rather different outline of the geological history in the central New Zealand region. He suggests the land connection between the North and South Islands during the lower Pliocene was more in the south Wellington area and that land was continuous along the southern Tararua Ranges, veering northeast before continuing north. He also illustrates the extensive marine transgression during the middle and late Pliocene which formed three straits: the 'Kuripapango Strait', between the rising Ruahine Range and the Kaweka Range-Kaimanawa Mountains; the 'Manawatu Strait', between the rising Ruahine and Tararua Ranges (Fig. 3.1B); and according to Stevens, the Cook Strait was formed in the upper Pliocene (~ 3 Ma) by deep scouring apparently along the 'Cook Strait Fault'. Stevens (1974) also suggested the Cook Strait was open until the Pleistocene when the sea level dropped 100 to 200 m. During this period (1.2 Ma – 500,000 years ago) evidence for land bridges between the Manawatu-Rangitikei

and Marlborough Sounds regions come from the presence of sedimentary rocks currently found in the d'Urville Island-Marlborough Sounds region. Paradoxically (and more recently), Lewis & Carter (1994) suggest that the formation of the Cook Strait occurred in the mid Pleistocene when the sea level rose causing tidal scour of an area termed 'the Narrows Basin'. Based on three unconformities representing erosion during interglacial periods and the presence of tidal flow during the last glacial maximum, Lewis and Carter suggest the Cook Strait first opened approximately 450,000 years ago; this is in contrast to Stevens' earlier (and probably incorrect) estimate of 3 million years.

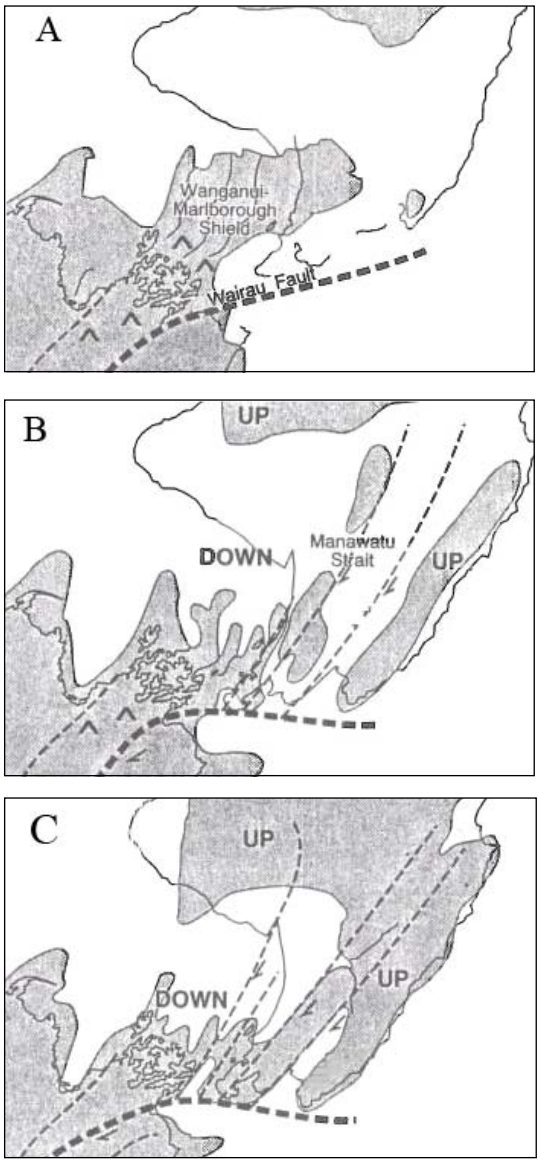

Figure 3.1. Palaeogeographic maps of the lower North Island (outline), showing land above sea level (grey) in the early Pliocene (A), end of the Pliocene (B), and in the early-mid Pleistocene (C) (modified from Lewis & Carter, 1994).

During the late Pliocene-early Pleistocene, the NI shoreline in the Wanganui region changed rapidly, an area known globally as the Wanganui Basin because it contains a continuous cyclostratigraphy since ca. 2.6 Ma (Naish, 1997). Uplifting of the Wanganui Basin has exposed foraminiferal and molluscan fossils at each of approximately 22 cyclotherms, which have been used to determine the paleobathymetry of the area (Naish & Kamp, 1997). As the northern part of the Wanganui Basin went through this neotectonic upwarping, the 'proto-strait' narrowed to form the 'Manawatu Strait', and then as land emerged from the sea to the east in the early-mid Pleistocene, the 'Manawatu Strait' was blocked and a 'proto-Manawatu River' formed (Fig. 3.1B-C) (Anderton, 1981; Cotton, 1958; Fleming, 1962).

The emergence date of the lower NI axial ranges is estimated at 1 M to ~ 500,000 years (Beu *et al.*, 1981; Ghani, 1978; Walcott, 1978). Beu *et al*. (1981), examined Nukumaruan (2.4 – 1.63 Ma) marine rocks, Mesozoic clasts, and the faulting patterns of Castlecliffian (1.63 – 0.34 Ma) rocks and inferred tectonic uplift of the Ruahine Ranges occurred mostly in the last 1 Ma. Uplift of the Tararua Ranges was more recent than the Ruahines with Ghani (1978), suggesting tectonic uplift around 500,000 – 200,000 years ago.

In conjunction with sea level changes and tectonism, volcanism also changed the landscape of the NI. Volcanism in the Taranaki region began in the early Pleistocene with the first volcano, Paritutu, radiometrically dated at around 1.75 Ma (Neall, 1974). Prior to this volcanic activity, the Taranaki region was below the sea surface and lahars produced from Paritutu flowed into the sea (Neall, 1982). The sequence of volcanic activity is referred to as the 'Taranaki Volcanic Succession' and the age of each volcano is as follows: Paritutu, last active around 1.76 Ma; Kaitake, last active 575,000 years ago; Pouakai, last erupted between 216,000 and 250,000 years ago; and finally Mt. Taranaki, last erupting only about 250 years ago (Neall, 1982). At the end of the Last Glacial Maximum (LGM) around 23,000 years ago, Mt. Taranaki erupted, causing a huge lahar avalanche which spread over 200 km^2 beyond the present coastline. This deposit (the Pungarehu Formation) joined the Farewell Rise, a spit formed by sand and gravel drifting northwards from the west coast of the South Island, resulting in a continuous landbridge between the North and South Island (Lewis & Carter, 1994).

At the other end of the NI, islands formed by volcanic eruptions were repeatedly connected to, and separated from, the mainland as a result of sea level changes during the glacial-interglacial cycles of the Pleistocene. For example, volcanic eruptions 5 - 6 Ma to the east of the Coromandel Peninsula produced the Mercury Islands and at least five separate domes formed in the vicinity of the current Alderman Islands; three were centred on Middle Island and remnants of two other domes are now known as Ruamahuanui Island and Hongiora Island (Homer & Moore, 1992). At the LGM (Fig. 3.2), the sea level was approximately 120 m lower than present (Carter et al., 1983). All the current New Zealand offshore islands were connected to the mainland except for the Three Kings

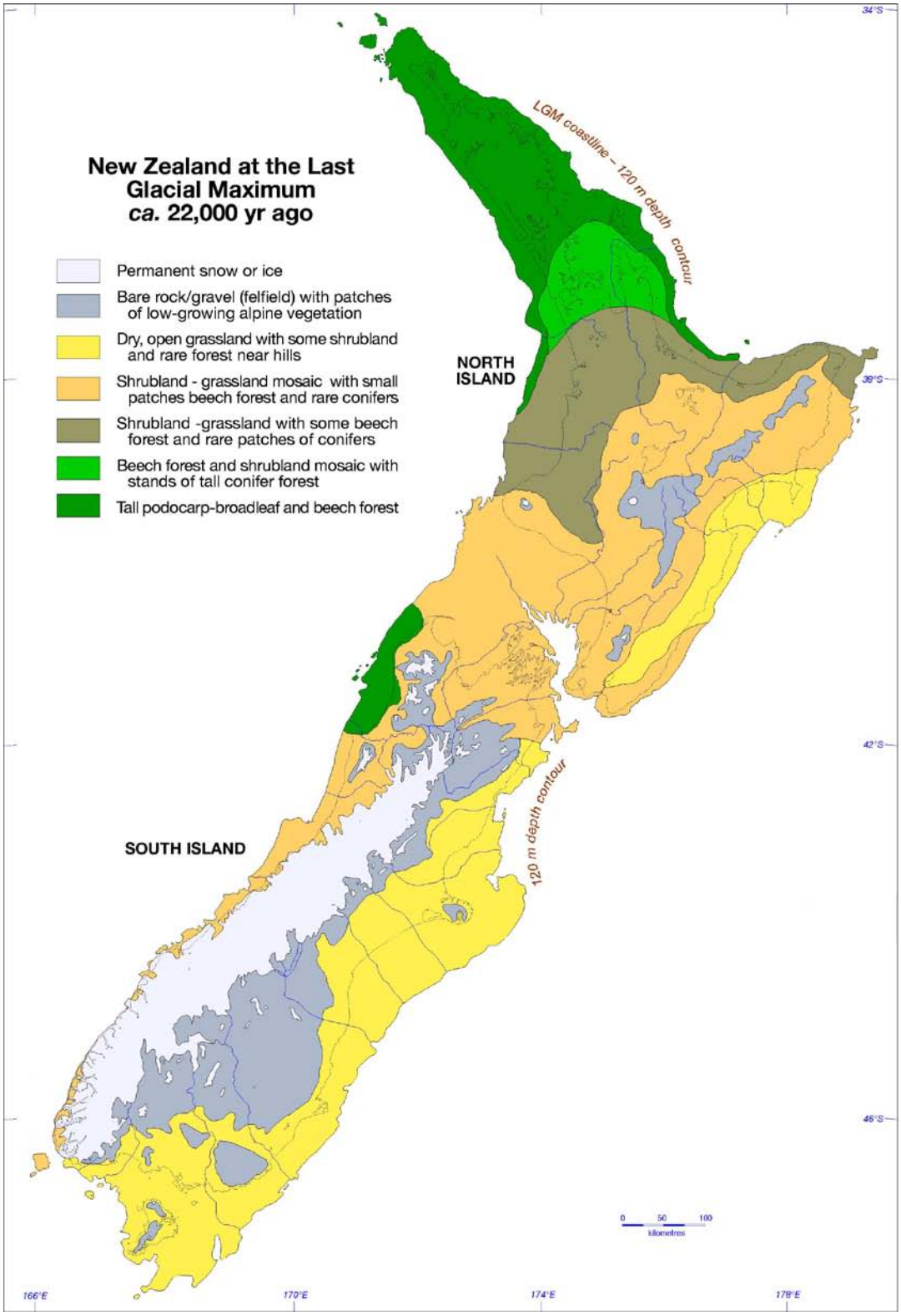

Figure 3.2. Map showing glaciers and vegetation zones in New Zealand at the Last Glacial Maximum (modified from Barrell *et al.*, 2005).

and the Poor Knights Islands (Hayward, 1986). The sea began to rise at a rate of about 10 m per 1000 years and by around 12,000 years ago, the Hen and Chickens, Great and Little Barriers, Cuvier and the Alderman Islands had become separated from the mainland. The majority of other islands, including the Mercury Islands, were separated between 10,000 – 7,000 years ago and the sea reached its present level 6,500 years ago (Gibb, 1983). The Mercury Islands separated from each other over several thousand years, first a 'super island' separated from the mainland Coromandel Peninsula and this became fragmented into Great Mercury, Red Mercury, Double, and Stanley Island with Middle, Green and Korapuki still connected to each other until around 6000 years ago (Towns *et al.*, 1990; Towns, 1994).

Not only has the NI undergone a changing coastline over the past 5 Ma as a result of volcanic eruptions and sea level changes, but volcanism in the central NI region has greatly affected the landscape. The Taupo Volcanic Zone (Healy, 1962) covers a distance of 300 km, from Ohakune to White Island in the Bay of Plenty. Over the past 5 Ma, almost 17,000 cubic kilometres of rhyolitic material has erupted within the zone (Healy, 1962). The last major eruption was only ~1850 years ago, which deposited pyroclastic rock over 20,000 km^2 of central NI and ash over 30,000 km^2 of eastern NI (Fig. 3.3A) (Wilson & Walker, 1985).

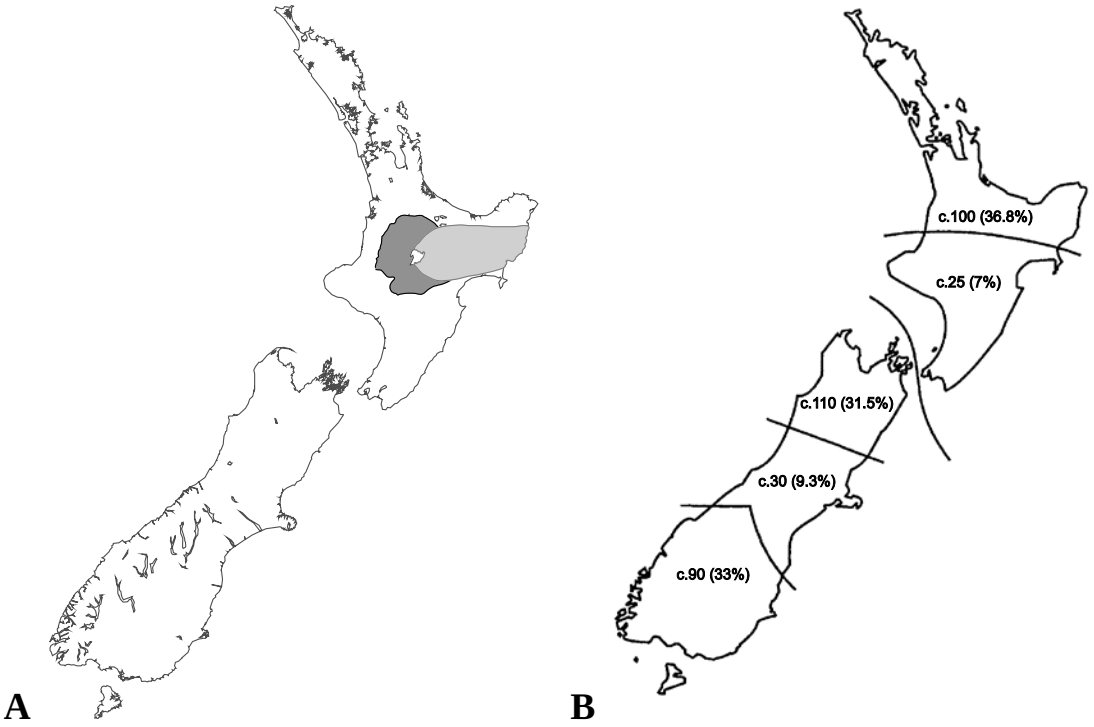

Figure 3.3. (A) Map showing the area of North Island covered by pyroclastic rock (dark grey) and ash (light grey) following the Taupo eruption ~1850 years ago. (B) Approximate numbers of endemic vascular plants according to Wardle 1963, and percentage of endemic insect species from Craw, 1988.

In addition to the geophysical history of NI, it is also interesting to note the different levels of biodiversity and endemicity of extant taxa throughout New Zealand. Based on the number of endemic vascular plants (Wardle, 1963) and endemic insect species (Craw, 1988), New Zealand has been divided into five regions of high and low endemicity (see Fig. 3.3B). There are three areas rich in endemic species – northern NI, northern SI, and southern SI, and two endemic poor areas - central SI and lower NI. The endemic rich northern NI region contains a range of invertebrates which are apparently restricted to the area above 39° latitude: for example, two species of stick insects, *Spinotechtrachus acornutus* and *Asteliaphasma jacunda* (Trewick & Morgan-Richards, 2005; Trewick *et al.*, 2008); a species of velvet worm, *Peripatoides aurorbis* (Trewick, 2000); three tusked weta, *Motuweta isolata*, *M. riparia*, and *Anisoura micobarica* (Gibbs, 2001); three giant

weta, *Deinacrida fallai, D. heteracantha* and *D. mahoenui* (Gibbs, 2001); and at least two ground weta, *Hemiandrus* "otekauri" and *H.* "elegans" (Johns, 2001). Likewise, there is greater species richness in the northern SI, for example five of the six short ovipositor ground weta species can be found in the northern half of SI (Johns, 2001). The classic explanation for these differing degrees of endemism is that areas of high endemism were glacial refugia (Dumbleton, 1970; Wardle, 1963) and the low endemicity in lower NI can be explained by marine transgression in the Pliocene (Rogers, 1989).

3.1.2 Objectives

Accordingly, the goal of this research is to determine the phylogeographical structure of endemic ground weta *H. pallitarsis* populations using mitochondrial cytochrome *c* oxidase subunit I (COI) sequences. Based on what is known about the geophysical history, patterns of endemicity, and current distribution of ground weta species in NI, several predictions can be made concerning the phylogeography of *H. pallitarsis*:

- *H. pallitarsis* has moved in a 'north to south' pattern. Weta would have been restricted to the northern NI, then dispersed south as land emerged above sea level in what is now the lower NI during the Plio-Pleistocene. Genetic diversity should be lower in the more recently colonized populations in the southern half of NI (e.g. Morgan-Richards *et al.*, 2001).

- *H. pallitarsis* has moved in a 'south to north' pattern due to the land connection between the (now) Wellington area and upper SI in the early Pliocene. Range expansion north would have occurred as land uplifted in the lower NI during late Pliocene – mid Pleistocene.

- Alternatively, any signal of the impact of Pliocene land formation on *H. pallitarsis* has been masked by more recent events such as habitat shifts during the

glacial-interglacial cycles in the Pleistocene, or the Taupo eruptions and subsequent ash and rock cover.

Overall, the main objective of this chapter is to evaluate how historical geophysical processes have influenced the phylogeographic patterns of *H. pallitarsis*. However, this chapter also aims to confirm the monophyly of weta putatively identified as *H. pallitarsis* from sites across the NI including on offshore islands, and also to assess the phylogeographic relationships and evaluate levels of population genetic partitioning within and among populations across NI, New Zealand.

3.2 Methods

3.2.1 Taxonomy of ground weta

Johns (1997) lists seven valid species within *Hemiandrus*, and Jewell (2007) described another two species from the Fiordland area. Johns (2001) added a further 28 undescribed species of *Hemiandrus*, the names of which he disclaimed as "not available" as per Article 8.3 of the International Code of Zoological Nomenclature (1999). Johns (2001) has given these undescribed species tag-names (in quotation marks), therefore I refer to the same tag-names.

3.2.2 Sampling sites and collections

A total of 176 *Hemiandrus pallitarsis* individuals were collected between November 2006 and April 2007 from 24 locations in NI, New Zealand (Table 3.1, Fig. 3.4). Specimens from seventeen mainland locations were available in the collection of Steve Trewick and Mary Morgan-Richards at the Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Palmerston North. Specimens from Great Barrier Island were provided by John Early at the Auckland Museum, and weta were collected from Little Barrier Island by the Department of Conservation (DoC) and donated to the Allan Wilson Centre. In total, nineteen mainland sites and five islands were sampled across NI. Weta were hand collected, identified and preserved as outlined in Chapter two. Specimens of *H. bilobatus*, *H.* "vicinus", *H.* "promontorius", *H.* "onokis" and *H.* "horomaka" were obtained as reference samples for phylogenetic out-grouping.

Table. 3.1. Sampling locations for *Hemiandrus* specimens. * Individuals used in Bayesian analysis of the entire COI gene fragment. † Locations above sea level during the early Pliocene (Fleming, 1979; Lewis & Carter, 1994) (see Fig. 3.7).

Species	Code	Locality	Coordinates	Accession No.
H. pallitarsis	GB	Great Barrier Island	36°18´ S, 175°32´ E	
H. pallitarsis	LW226*	Little Barrier Island	36°11´ S, 175°32´ E	
	LW227*			
H. pallitarsis	CU76	Cuvier Island †	36°26´ S, 175°46´ E	
	CU77*			
	CU79			
	CU81			
	CU82*			
	CU83			
	CU84			
	CU85			
	CU86			
H. pallitarsis	MI95*	Middle Island, Mercury Islands †	36°38´ S, 175°51´ E	
	MI96			
	MI97			
	MI98*			
	MI99			
	MI100			
	MI101			

	MI102		
	MI103		
H. pallitarsis	AL104	Ruamahuanui Island, Alderman Islands †	36°57´ S, 176°05´ E
	AL105*		
	AL106*		
	AL107*		
	AL108		
	AL110		
	AL111		
	AL112		
H. pallitarsis	CO71	Mt Moehau, Stony Bay †	36°30´ S, 175°25´ E
	CO72		
	CO73		
	CO74		
	CO75		
	CO144		
	CO145*		
	CO148		
	CO149*		
	CO151		
	CO161		
H. pallitarsis	CO53	Taiharuru Bay †	36°36´ S, 175°33´ E
	CO61		
	CO62		

	CO66		
	CO67		
H. pallitarsis	CO37	Coromandel Town †	36°44´ S, 175°30´ E
	CO41		
	CO121		
	CO127		
	CO128		
	CO129		
	CO130		
	CO141		
H. pallitarsis	KA87	Kauraeranga Valley †	37°07´ S, 175°37´ E
	KA88*		
	KA89		
	KA90*		
	KA91		
	KA94		
	KA119		
	KA120		
H. pallitarsis	GW199	Opepe Memorial Reserve, Taupo †	38°46´ S, 176°13´ E
	GW200		
H. pallitarsis	LW189	Lake Waikaremoana †	38°45´ S, 177°09´ E
	GW230		
	GW231		
	GW66*		EU676740

H. pallitarsis	GW91*	Mt Holdsworth, Wairarapa	40°55´ S, 175°20´ E	EU676768
H. pallitarsis	PN22	Parata Street, Palmerston North	40°21´ S, 175°37´ E	
	PN23			
	PN178			
H. pallitarsis	GW92A	Victoria Esplanade, Palmerston North	40°22´ S, 175°36´ E	
H. pallitarsis	PN174*	Bledisloe Park, Palmerston North	40°22´ S, 175°37´ E	
	PN175*			
	PN176*			
	PN177*			
H. pallitarsis	GW232	Turitea Road, Palmerston North	40°21´ S, 175°37´ E	
H. pallitarsis	GW87*	Totara Reserve, Pohangina Valley	40°08´ S, 175°50´ E	
H. pallitarsis	GW190	Gordon's Bush, Wanganui	39°54´ S, 175°05´ E	
H. pallitarsis	GW171	Cambridge †	37°53´ S, 175°28´ E	
H. pallitarsis	TK187*	Oakura Beach, Taranaki	39°07´ S, 173°57´ E	
	GW228			
	GW235*			
	GW236			
H. pallitarsis	GW89A	Khandallah, Wellington †	41°14´ S, 174°47´ E	
	GW89B			
H. pallitarsis	GW80	Newlands, Wellington †	41°13´ S, 174°49´ E	
	GW82			
	GW84			

	GW176*			
	GW97*			EU676797
H. pallitarsis	WN181	Otari-Wilton Bush, Wellington †	41°16´ S, 174°45´ E	
	WN182			
	WN183			
H. bilobatus	GW240*	Botanical Gardens, Wellington †	41°16´ S, 174°46´ E	
	GW241*			
H. bilobatus	GW25*	Newtown, Wellington †	41°18´ S, 174°46´ E	EU676794
H. "onokis"	GW127*	Springfield, Porters Pass †	43°20´ S, 171°55´ E	EU676778
	GW129*			EU676779
H. "horomaka"	GW120*	Akaroa, Banks Peninsula †	43°48´ S, 172°58´ E	EU676771
H. "promontorius"	GW55*	Marfell's Beach, Cape Campbell, South Marlborough †	41°43´ S, 174°12´ E	EU676789
	GW122*			EU676777
H. "promontorius"	GW193*	Muritai Reserve, Seaview, South Marlborough †	41°38´ S, 174°08´ E	
H. "vicinus"	GW54*	Whites Bay Track, Blenheim †	41°31´ S, 173°57´ E	EU676788

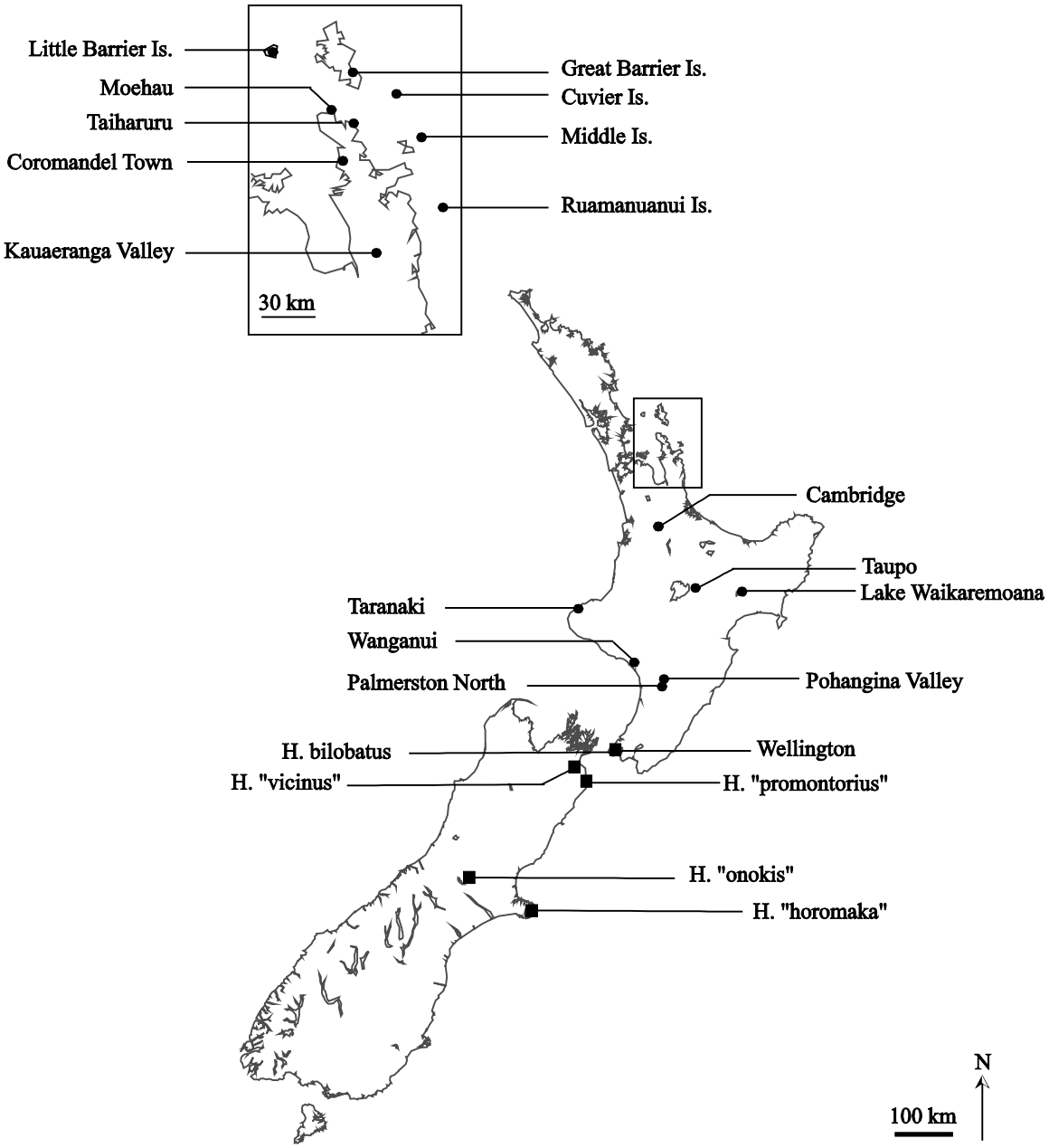

Figure 3.4. Collection locations of *Hemiandrus pallitarsis* (●), and outgroup species (■) in New Zealand. Inset shows collection locations in the Coromandel.

3.2.3 DNA extraction

Muscle tissue from the hind femur of one leg of each specimen was dissected out and stored at -20°C. Total genomic DNA was extracted using a salting-out extraction method (Sunnucks & Hales, 1996). Tissue was crushed in 10µL of 10mg/mL proteinase K and incubated in 600µL of TNES buffer [20mM EDTA

(ethylenediaminetetraacetic acid), 50mM TrisHCl, 400mM Sodium Chloride (NaCl), 0.5% Sodium Dodecyl Sulphate (SDS] at 55°C for 1-3 hours. 170 µL of 5M NaCL was added and the tube was mixed well for 15 seconds. The tube was then centrifuged at 13000 revolutions per minute (rpm) for 5 min in a Heraeus benchtop centrifuge. 780µL of supernatant was removed and mixed with 780µL of ice cold 100% ethanol and allowed to stand for approximately one hour or overnight at -20°C. The samples were then centrifuged for a further 5 mins at 13000 rpm. Ethanol was removed from the tube and the DNA was further washed with 400µL of 70% ethanol and then centrifuged for 5 mins at 13000 rpm. The ethanol was then removed and the DNA allowed to air dry before it was re-suspended in 50µL of Milli-Q water. Extractions were quantified using NanoDrop (3.3.0) and agarose gel electrophoresis.

3.2.4 PCR amplification

A range of universal invertebrate primer combinations were tried in order to sequence the COI and COII genes of the mitochondrial genome. Where possible, a ~1400 base pair (bp) fragment comprising most of the COI gene was amplified using the following primers (Folmer *et al.*, 1994) (Fig. 3.5):

- LCO1490: 5'-GGTCAACAAATCATAAACATATTGG
- L2-N-3014: 5'- TCCAATGCACTAATCTGCCATATTA

A smaller ~700 bp region of the COI gene was amplified using the primer pair (Folmer *et al*., 1994) (Fig. 3.5):

- LCO1490: 5'-GGTCAACAAATCATAAACATATTGG
- HCO2198: 5'- TAAACTTCAGGGTGACCAAAAAATCA

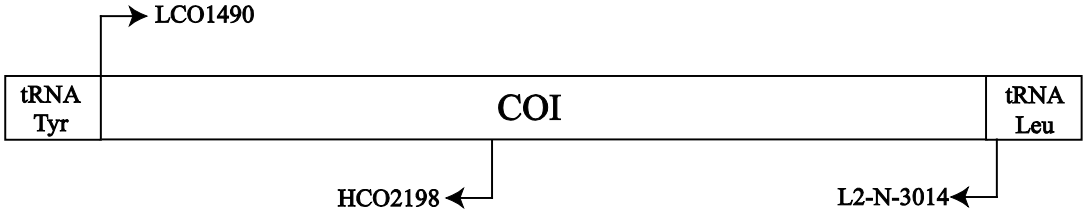

Figure 3.5. Diagram of the cytochrome oxidase I gene showing positions and sequencing directions of the primers used in this study.

In addition, fragments from five outgroup species (731 - 847 bp) were used in the analyses (made available by R. Pratt). These fragments overlapped at the 3' end of the COI gene with the ~1400 bp fragments sequenced in this study, forming overall fragments of length 1432 bp (missing nucleotides at 3' end of some sequences and 5' end of outgroup sequences).

Polymerase chain reaction (PCR) used the following conditions: 94°C for 2 min (initial denaturation); 38 cycles (35 cycles for the smaller fragment) of 94°C for 30s (denaturation), 52°C for 30s (annealing), and 72°C for 1m 30s (primer extension); 72°C for 8 min (final extension). Amplification reactions consisted of 1.0µl of the extracted DNA, 0.08µl of ABGene Taq DNA polymerase (500U), 0.8µl of 25mM $MgCl_2$, 0.2 µl of each 2mM dNTP, 0.4µl of each 10µM primer, 1.0µl of 10x PCR buffer, and 5.82µl of Milli-Q water in 10 µl total volume. Agarose gel electrophoresis was used to assess the quantity and quality of the amplified DNA products.

3.2.5 DNA sequencing

PCR products of the expected size were purified for sequencing using the SAP/EXO1 digest protocol (USB Corp., Cleveland, OH, U.S.A.). Incubation of PCR products and SAP/EXO1 enzymes was at 37ºC for 30 min followed by a denaturation step of 80ºC for 15 min. Purified PCR products were sequenced with one or more of the primers listed above, using BigDye™ Terminator chemistry (Perkin-Elmer Applied Biosystems) (thermal cycling conditions were: 26 cycles of 96ºC for 10 s, annealing at 50ºC for 5 s and 60ºC for 4 mins). Sequencing products were cleaned following the CleanSEQ® Dye Terminator Removal procedure (Agencourt Bioscience Corp.). 10µl of CleanSEQ® magnetic beads and 62µl of 85% ethanol were added to the samples and mixed. Samples were incubated on a magnetic plate for 3-5 min before the supernatant was aspirated, then 100µl of 85% ethanol was added and aspirated. After 10 min of air-drying off the magnetic plate, samples were returned to the plate, 40µl of elution buffer (0.1mM EDTA) was added and the plate was incubated for 5 min to separate the magnetic beads from the solution. 20µl of solution from each sample was then submitted to the Allan Wilson Centre Genome Service, Massey University, Palmerston North for capillary separation on an AB13730 genetic analyser (Applied Biosystems Inc.). DNA sequence reads were aligned with SEQUENCHER v. 4.2 (Gene Codes

Corp., Michigan). Ambiguous base calls were corrected manually and ambiguous end regions were trimmed so that all individuals were analysed over the same sequence length (638 bp for the shorter fragment and 1432 bp for the longer fragment). Unique haplotypes were identified using the program MacClade (v. 4.0).

3.2.6 Nuclear copies

Nuclear copies of mitochondrial genes, often referred to as pseudogenes or *Numts*, occur when DNA, homologous to mtDNA is integrated into the nuclear genome (Du Buy & Riley, 1967). In such situations, PCR can sometimes amplify the *Numt* in addition to, or in preference to, the mitochondrial gene region of interest which then leads to incorrect genetic phylogenies. *Numts* have been found to have derived from all parts of the mtDNA genome, vary in size and vary in the levels of similarity to their mitochondrial counterparts (Zhang & Hewitt, 1996). As of 2001, *Numts* have been documented in over 82 different species (for a list of all species see http://www.pseudogene.net), and appear to be especially common in Orthoptera (Bensasson *et al.*, 2001). Previous research on ground weta incurred problems with amplifying nuclear copies within the COI gene of ground weta and in particular *H. pallitarsis* (pers. comm. Renae Pratt). Consequently, in order to avoid the inclusion of nuclear sequences into the dataset a range of precautions were enforced: firstly, PCR amplifications showing more than one band were discarded; secondly, stock solutions of extracted DNA were serially diluted in order to reduce the ratio of nuclear DNA to mtDNA, and therefore, favour the amplification of mitochondrial targets as opposed to nuclear targets (Kidd & Friesen, 1998); thirdly, nucleotide sequences sequenced from both ends of the gene fragment but yielded contig ambiguities were discarded; fourthly, sequences containing unexpected insertions or deletions, stop codons or frameshifts were also discarded (Kidd & Friesen, 1998); and finally, a primer sequence was developed from a suspected *Numt* sequence (PCR product clearly showing double bands; see Appendix E) and used to amplify possible *Numts* from several locations. The resulting sequences were analysed with several sequences not suspected of being nuclear copies using Neighbour-joining (NJ) method in PAUP* v.4.0b10 (Swofford, 2002). The NJ tree shows the suspected *Numts* form a clade which is sister to the rest of

the sequences (Fig. 3.6). This information was used to identify putative *Numt* sequences.

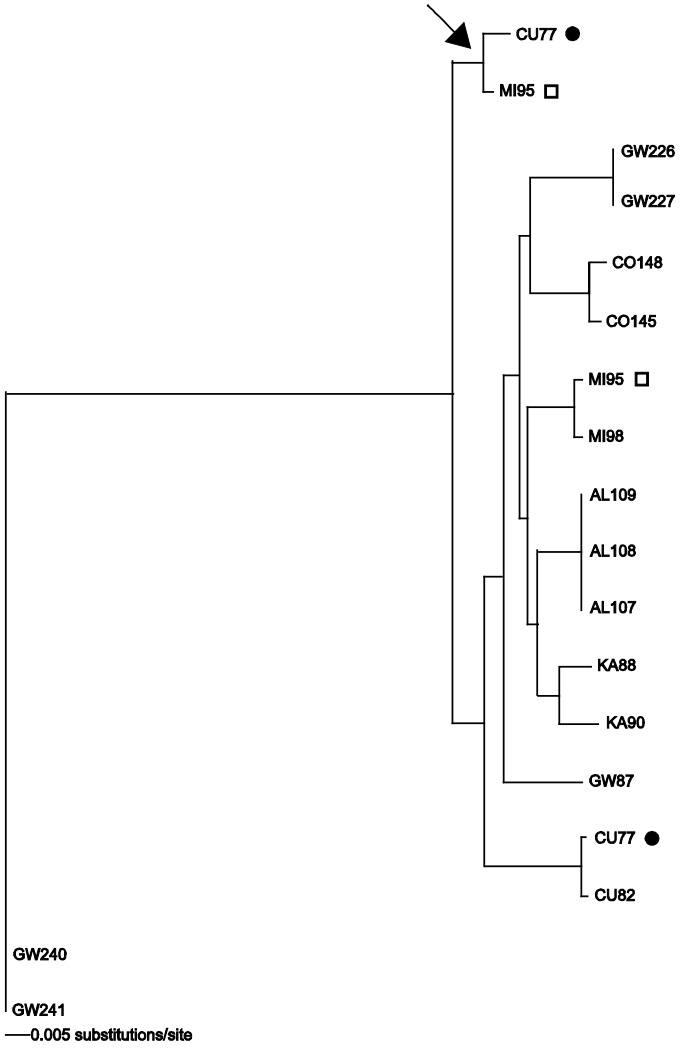

Figure 3.6. Neighbour Joining (NJ) tree with *H. bilobatus* as outgroup. Symbols indicate alternative mtDNA COI sequences from the same individuals: ● Cuvier Island, □ Middle Island. Arrow shows position of sequenced *Numts*.

3.2.7 Phylogenetic analyses

Phylogenetic analyses were performed using neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) methods implemented by PAUP* v.4.0b10 (Swofford, 2002). MP reconstructions were unweighted and run using the default settings. To determine the most appropriate model of DNA evolution, log-likelihood scores were generated and used to conduct an Akaike information criterion

(AIC) test in MODELTEST 3.06 (Posada & Crandall, 1998). NJ and ML reconstructions were run with the model and parameters obtained using MODELTEST 3.06. Bootstrap resampling with 1000 replicates was used to determine the degree of support for each node in MP and NJ trees. Due to computing-time constraints only 100 replicates were used for the ML tree in conjunction with a cut down taxon list, which included only one representative sequence for each clade. Bayesian analysis was implemented with MR BAYES v.3.1.2 (Ronquist & Huelsenbeck, 2005) using HKY (nst=2) or GTR (nst=6) models, and were run for 6 million generations with two independent simultaneous runs with three heated chains that were sampled every 1000 generations. Consensus tree and credibility values for each node were obtained from sampled trees after burning the first 20% of the chain.

To investigate the monophyly of *H. pallitarsis*, larger COI fragments (1432 bp) were analysed, with ten individuals from five of the closest known relatives of *H. pallitarsis* included as outgroups (based on ovipositor length and modification of the 6th abdominal sternite; Johns 2001). Seven outgroup sequences and three *H. pallitarsis* sequences, from the 3' end of COI gene, were provided by R. Pratt. Intraspecific phylogenetic analyses were performed using the smaller COI fragment (638 bp).

3.2.8 Population genetic analyses

Nucleotide diversity (π, Tajima, 1983), haplotype diversity (*h*, Nei, 1987), estimate of effective population size (θ(S), Tajima, 1989), and Tajima's test of selective neutrality (Tajima's *D*: Tajima, 1989) were calculated for each location and across all samples in the program ARLEQUIN 2.0 (Excoffier *et al.*, 2005). The partition of genetic variability among populations and among groups of populations was defined by analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) using ARLEQUIN 2.0. In order to determine the possible origin if diversification of *H. pallitarsis*, thirteen populations were partitioned into those regions above sea level (Region 1 in northern NI and Region 3 in the Wellington area) and below sea level (Region 2) during the Pliocene (Fig. 3.7). Greater genetic diversity occurs in older populations due to *in situ* survival and only a subset of this diversity is carried into founding populations from the source population (Avise, 2000; Hewitt, 1999). Therefore, we would expect to find greater genetic diversity in Region 1 based on the prediction of 'north to south'

expansion, or in Region 3 based on the 'south to north' prediction (see Introduction - *3.1.2*).

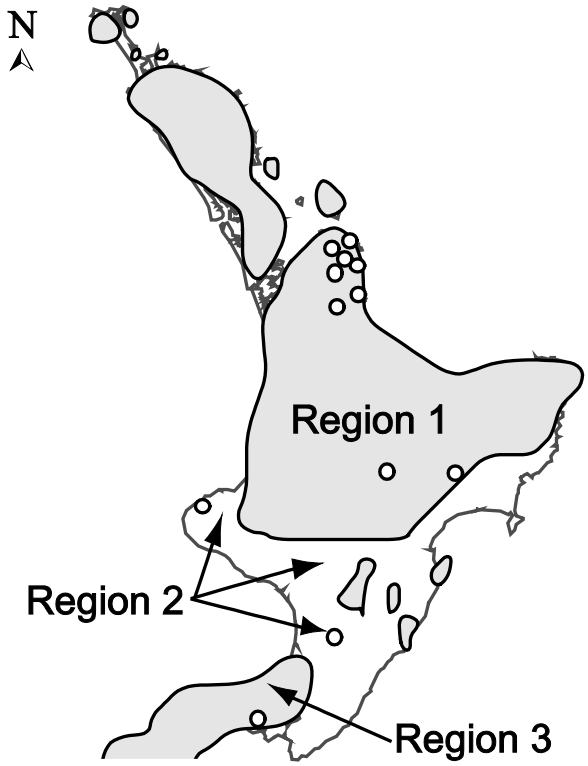

Figure 3.7. Map showing hypothesized distribution of land in the North Island at the early Pliocene (grey areas) (modified from Morgan-Richards *et al.*, 2001). Sampling locations within each region are indicated by white circles.

Pairwise estimates of genetic differentiation between populations were examined using Φ_{ST}, an analogue of F_{ST} that incorporates haplotype frequency and similarity (Excoffier et al. 1992). Genetic distances used were uncorrected *p* distances. Euclidean geographical distances among all population pairs were measured in Le Progiciel. Pairwise matrices of geographical distances and Φ_{ST} were compared using Mantel Tests for matrix correlation (Mantel, 1967), with significance assessed by 10,000 randomizations of the genetic distance matrix. Separate Mantel Tests were performed at three different geographic scales: the first and smallest geographic area encompassed the Coromandel Peninsula and nearby offshore islands, including Great Barrier and Little Barrier Islands (Fig. 3.8A); the next scale included the previous area plus the

central and eastern NI (i.e. Cambridge, Taupo and Lake Waikaremoana) (Fig. 3.8B); and the last Mantel Test covered the entire sampling area (Fig. 3.8C). These isolation-by-distance (IBD) analyses were performed using ARLEQUIN 2.0 (Excoffier *et al.*, 2005).

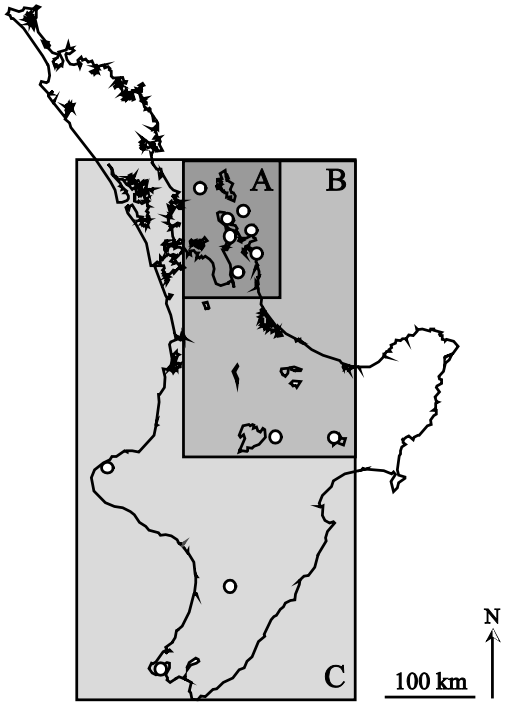

Figure 3.8. Map of NI, New Zealand showing populations sampled at three geographical scales: A) Coromandel Peninsula and offshore islands (dark grey); B) Taupo, Lake Waikaremoana and populations in A (medium grey); C) Taranaki, Palmerston North, Wellington and populations in A and B (light grey).

3.2.9 Molecular dating

A molecular clock calibration rate of between 1.4 – 2.6% sequence divergence per million years was used to estimate possible divergence times of lineages within *H. pallitarsis*. This range of calibration rates has been derived from comparisons between geological data and arthropod mitochondrial data (Brower, 1994; Brown *et al.*, 1996; Brown *et al.*, 1979; Folmer *et al.*, 1994; Juan *et al.*, 1995; Knowlton *et al.*, 1993; Knowlton & Weigt, 1998).

3.3 Results

Two datasets of cytochrome oxidase DNA sequences were produced for the ground weta. The first dataset contained sequences from 24 individuals of *H. pallitarsis* from 13 locations, and five outgroup species: two *H.* "onokis" individuals, one specimen of *H.* "horomaka", three *H. bilobatus* individuals, three *H.* "promontorius" individuals, and one specimen of *H.* "vicinus" (Table 3.1). The dataset contained 1432 bp, of which 406 (28.4%) were variable, and 353 (24.7%) were parsimony-informative. Approximately 84% of all substitutions were at third-codon positions, 13% of the variable sites occurred at the first-codon position, and 2% were found at the second-codon position.

The second dataset contained sequences from a further 64 *H. pallitarsis* individuals from 6 additional locations. In total, the second dataset contained sequences from 88 *H. pallitarsis* individuals from 18 locations, each 638 bp in length. The 638 bp COI alignment contained 131 variable sites (20%), of which 117 (18%) were parsimony informative. Approximately 89% of all substitutions were at third-codon positions, and only 10% of the variable sites occurred at the first-codon position; no substitutions were found at the second-codon position. There was no evidence of frameshifts or stop codons in the amino acid translation. All but two substitutions were synonymous mutations resulting in no amino acid change: two individuals from Middle Island and one from Cuvier Island contained Isoleucine amino acid instead of Valine; and all the northern Coromandel Peninsula individuals (i.e. Moehau, Taiharuru and Coromandel Town) contained Threonine amino acid instead of Alanine. Fifty-five unique haplotypes were detected, with no haplotype sharing among sampling sites (Appendix F). The largest number of nucleotide substitutions between haplotypes was 57 (8.9% sequence divergence), between a haplotype found in Taupo and one found in Wanganui, and only a single base pair substitution separates the nearest haplotypes (0.16% sequence divergence).

3.3.1 Phylogenetic analyses

Phylogenetic analysis of the long DNA fragment (1432 bp) using Bayesian search method produced the consensus tree shown in Figure 3.9. The 24 *H. pallitarsis*

individuals formed a monophyletic group (posterior probability = 0.98), which was sister to the Canterbury and Banks Peninsula species (*H.* "onokis" and *H.* "horomaka", respectively). *H. bilobatus* from Wellington forms a clade with *H.* "promontorius" from Cape Campbell in the north east South Island. The dominant split within the *H.* "promontorius" clade equates to two separate collection sites, Marfell's Beach and Muritai Reserve, which are approximately 26km from each other. The Marlborough sounds species (*H.* "vicinus") is sister to Wellington and Cape Campbell species (*H. bilobatus* and *H.* "promontorius"). Although each species appears to form a monophyletic clade (despite the paucity of sampling of the outgroup taxa), there is greater diversity within *H. pallitarsis* than there is between *H. bilobatus* and *H.* "promontorius" and between *H.* "onokis" and *H.* "horomaka".

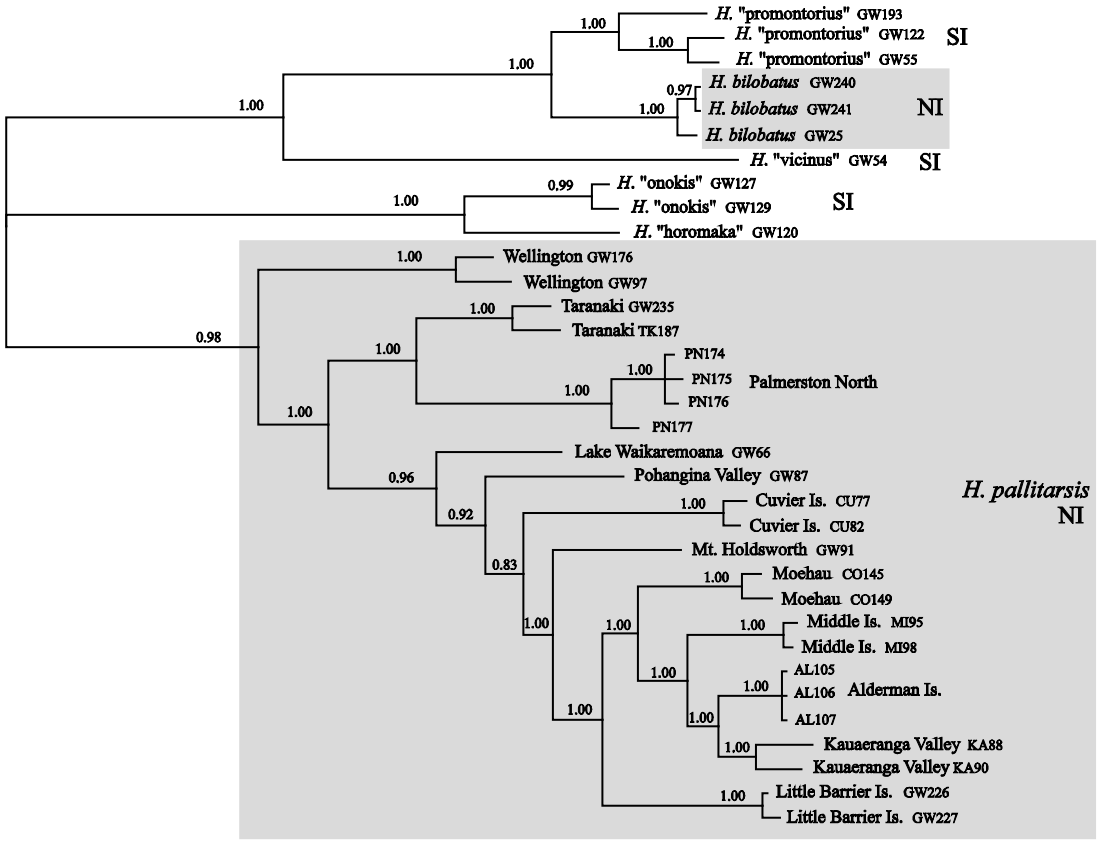

Figure 3.9. Bayesian consensus network showing the evolutionary relationships among short-ovipositor ground weta of New Zealand: *Hemiandrus pallitarsis* (large grey box), *H. bilobatus*, *H.* "onokis", *H.* "horomaka", *H.* "promontorius" and *H.* "vicinus". Bayesian analysis based on 1432 bp of the COI mitochondrial gene, implemented with MrBayes v3.1.2. (Ronquist & Huelsenbeck, 2005) using HKY (nst=2) model, consisting of 6 million generations with two independent, simultaneous runs with three heated chains and a sample frequency of 1000 generations, and a 10% burn in. Node stability is indicated by posterior probabilities.

All NJ, MP (consensus of 635 trees), ML (single optimal tree, -ln *L* = 2619.5477) and Bayesian analyses of the 55 haplotypes with the short dataset (638 bp) resulted in trees with similar topologies (Fig. 3.10, Fig. 3.11) The topology found was similar to that indicated by the Bayesian analysis of the long dataset (Fig. 3.9). All analyses revealed three major clades within *H. pallitarsis*: a predominantly northern clade (Clade I) (with the inclusion of Palmerston North individuals, Pohangina Valley and Wanganui), a central clade containing some Palmerston North, Taranaki, Taupo and Cambridge individuals (Clade II), and a southern (Wellington) clade (Clade III). Bootstrap resampling with NJ, MP and ML, and the Bayesian credibility values all indicated strong support for the three clades. Palmerston North was the only population which has haplotypes that occur in two clades; individuals from the northern side of the Manawatu River yielded Clade I while individuals from the south side of the river yielded Clade II haplotypes (there was no correlation with collector or time of sequencing). These two populations are separated by < 7 kms but are genetically separated by between 6.3 – 7.7% sequence divergence (see Results - 3.3.4). Internal edges within Clade I are short implying little variation at this level; these short edges correspond to areas of conflict within the Bayesian consensus network (Fig. 3.11). Within Clade I, genetic structure is concordant with geographic distribution; populations from the northern half of the Coromandel Peninsula (i.e. Moehau, Taiharuru and Coromandel Town) are sister to Kauraeranga Valley, at the base of the peninsula, Middle Island and Ruamahuanui Island. However, Cuvier Island is not part of this Coromandel clade, bootstrap resampling supports the placement of an individual from Wanganui as sister to weta from Cuvier Island. There is also strong support for the separation of populations within Wellington (Clade III) - individuals from the suburb Newlands are sister to individuals from the suburbs Khandallah and Wilton and differ from each other by < 5.3%, although they are only ~ 7 km apart.

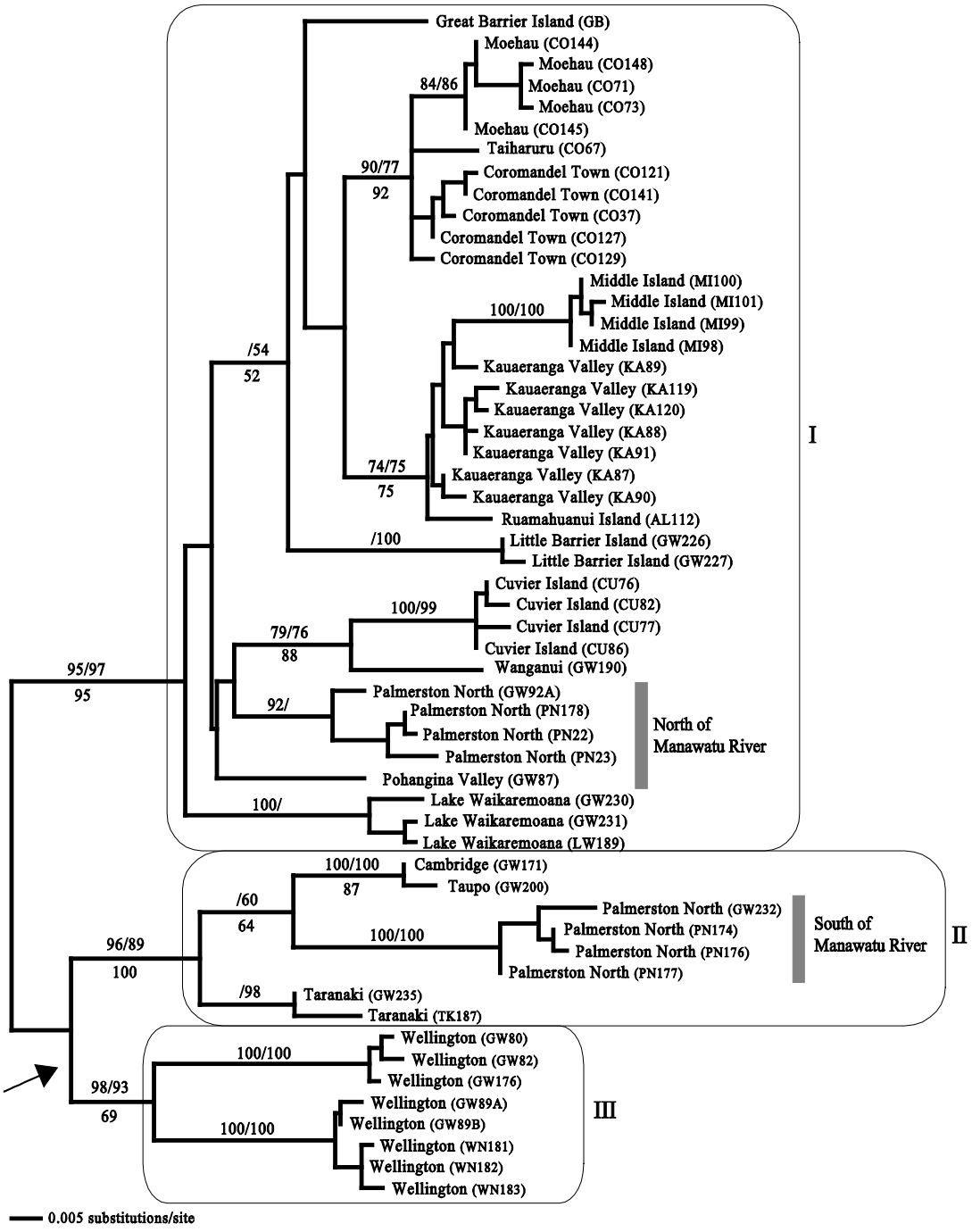

Figure 3.10. Maximum likelihood tree for *Hemiandrus pallitarsis* based on 638 bp of the COI mitochondrial gene. Tree is midpoint rooted due to long branch lengths of outgroup species. An arrow indicates position of root in analyses that include outgroup *Hemiandrus* spp. Modeltest v3.06 selected TVM + I + G model (-ln *L* = 3057.3407), with a gamma distribution shape parameter of 0.5777, 0.5548 proportion of invariable sites, and base frequencies of A = 0.26410, C = 0.25660, G = 0.16600, and T = 0.31330. Three measures of branch support are indicated with 1000 NJ and MP bootstrap replicates shown above edges, respectively, and 100 ML bootstrap replicates shown below edges. Terminal labels indicate sampling location and specimen code.

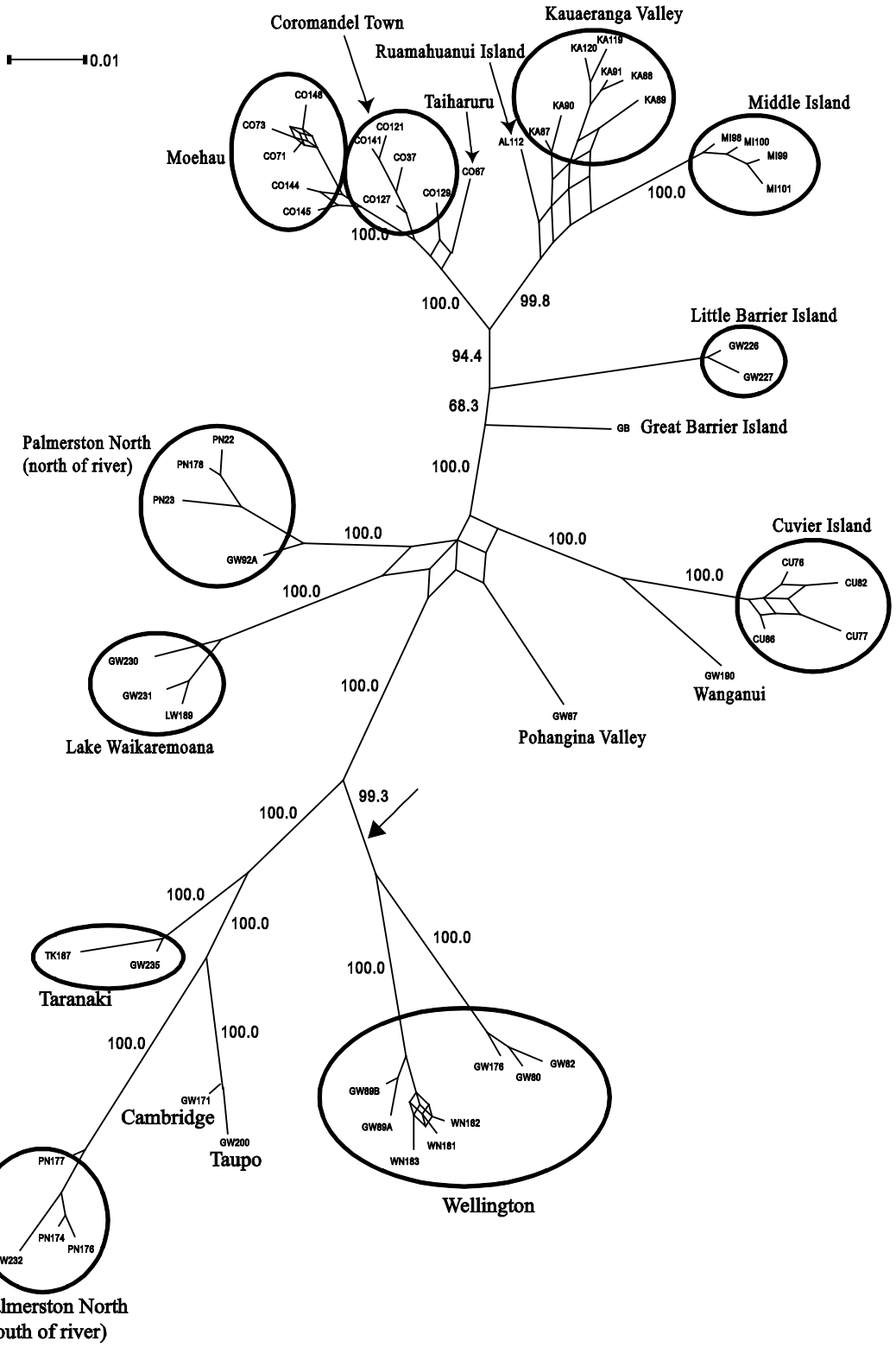

Figure 3.11. Unrooted consensus network for *Hemiandrus pallitarsis* COI mtDNA sequences. Bayesian analysis implemented with MrBayes v3.1.2. (Ronquist & Huelsenbeck, 2005) using HKY (nst=2) model, consisting of 6 million generations with two independent, simultaneous runs with three heated chains and a sample frequency of 1000 generations, and a 20% burn in. Values on branches are Bayesian credibility values. Terminal labels indicate individual codes and sampling locations are indicated by ellipses.

3.3.2 Population genetic analyses

Of the 55 COI haplotypes sequenced, 51 were used for the population genetic analyses. Four of the populations were removed as they each had a sample size of one (Great Barrier Island, Cambridge, Wanganui and Pohangina Valley). Tajima's *D* was not significantly different from zero in any population (Table 3.2) indicating no evidence for selection on this locus (Tajima, 1989). Nucleotide diversity (π) ranged from 0 – 0.0429 (mean = 0.0086, SD = 0.0054) with Palmerston North having the highest measure of nucleotide diversity (Table 3.2). Haplotype diversity (*h*) ranged from zero, where each individual in the population has the same haplotype, to one, where each individual in the population has a unique haplotype (mean = 0.6316, SD = 0.1393). There were three locations in which only a single haplotype was found at each site, resulting in no haplotypic variation ($h = 0$): Taiharuru (one haplotype/five individuals), Ruamahuanui Island (one haplotype/eight individuals), and Taupo (one haplotype / two individuals). Two populations had a haplotype diversity of one: Little Barrier Island (two haplotypes / two individuals) and Lake Waikaremoana (three haplotypes / three individuals). Overall, there is a pattern of low nucleotide diversity and high haplotype diversity for this COI gene fragment in *H. pallitarsis*.

The 13 populations were partitioned into those regions above sea level in the early Pliocene (Regions 1 in the northern half of NI and Region 3 in the Wellington area) and the area below sea level (Region 2) (Fig. 3.7) to determine the possible origin of diversification of *H. pallitarsis*. Higher diversity was found in Region 3 (Wellington), followed by Region 2 (below sea level) and populations now occupying areas of substantial land area during the Pliocene in the northern half of the NI had the lowest haplotype and nucleotide diversity (Table 3.2).

Overall genetic structure was high. AMOVA revealed that 73 of 78 (93.6%) pairwise population Φ_{ST} comparisons were statistically significant ($P \leq 0.05$), and Φ_{ST} values were relatively high, with a mean of 0.829 (range 0.42 – 1.00) (Table 3.3). AMOVA also revealed 79.4% of the molecular variance was partitioned among populations while only 20.6% ($P \leq 0.001$) was partitioned within populations (Table 3.4).

Collection Location (no. of populations sampled)	*N*	No. of haplotypes (*K*)	No. of polymorphic sites *S*	Haplotype diversity *h* (SD)	Nucleotide diversity π (SD)	Theta S θ (SD)	Tajima's D	Haplotype ID (no. of individuals)
Great Barrier Island* (1)	1	1	0	NA	NA	NA	NA	A1
Little Barrier Island (1)	2	2	2	1.000 (0.500)	0.0032 (0.0039)	2.000 (1.7320)	0.0000	A2, A3
Moehau (2)	8	5	7	0.7424 (0.1158)	0.0036 (0.0024)	2.3180 (1.1998)	-0.0764	A4(2), A5(2), A6(2), A7, A8
Cuvier Island (1)	9	4	5	0.5833 (0.1833)	0.0023 (0.0017)	1.8397 (1.0681)	-0.9100	A9(6), A10, A11, A12
Taiharuru (1)	5	1	0	0.0000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.0000	A13(5)
Middle Island (1)	9	4	3	0.5833 (0.1833)	0.0013 (0.0012)	1.1038 (0.7385)	-0.9361	A14(6), A15, A16, A17
Coromandel Town (2)	8	5	9	0.8929 (0.0858)	0.0063 (0.0040)	3.4711 (1.8179)	0.7968	A18(2), A19, A20, A21(2), A22(2)
Ruamahuanui Island (1)	8	1	0	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.0000	A23
Kauraeranga Valley (2)	8	7	14	0.9643 (0.0772)	0.0082 (0.0051)	5.3994 (2.6556)	-0.1409	A24, A25, A26, A27, A28, A29, A30(2)
Cambridge* (1)	1	1	0	NA	NA	NA	NA	B40
Taupo (1)	2	1	0	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.0000	B41(2)
Lake Waikaremoana (1)	3	3	10	1.000 (0.2722)	0.0104 (0.0085)	6.6667 (4.3278)	-0.0002	A31, A32, A33
Taranaki (1)	4	2	6	0.500 (0.2652)	0.0047 (0.0037)	3.2727 (2.0875)	-0.8086	B42, B43(3)
Wanganui* (1)	1	1	0	NA	NA	NA	NA	A34
Palmerston North (4)	9	8	56	0.9722 (0.0640)	0.0429 (0.0236)	20.6045 (8.8897)	1.6763	A35, A36, A37, A38, B44, B45(2), B46, B47
Pohangina Valley* (1)	1	1	0	NA	NA	NA	NA	A39
Wellington (3)	9	8	37	0.9722 (0.0640)	0.0292 (0.0163)	13.6137 (5.9922)	1.6903	C48(2), C49, C50, C51, C52, C53, C54, C55

Region 1	**64**	**35**	**50**	**0.5766 (0.1418)**	**0.0036 (0.0027)**	**2.2798 (1.3538)**	
Region 2	**15**	**12**	**62**	**0.7361 (0.1646)**	**0.0238 (0.0137)**	**11.9386 (5.4886)**	
Region 3	**9**	**8**	**37**	**0.9722 (0.0640)**	**0.0292 (0.0163)**	**13.6137 (5.9922)**	
Total (25)	**88**	**55**	**149**	**0.6316 (0.1393)**	**0.0086 (0.0054)**	**4.6377 (2.3470)**	**1.2912**

Table 3.2. Collection locations, number of individuals collected, molecular indices and haplotype codes. * removed from population-level analyses due to sample size of one.

Table 3.3. Pairwise Φ_{ST} values among populations of *Hemiandrus pallitarsis*. Comparisons that were significant at the $P < 0.05$ level are indicated by an asterisk.

Population	1	2	3	4	5	6	7	8	9	10	11	12
1 Little Barrier Is.												
2 Moehau	0.930*											
3 Cuvier Is.	0.958*	0.930*										
4 Taiharuru	0.987*	0.876*	0.970*									
5 Middle Is.	0.970*	0.925*	0.963*	0.974*								
6 Coromandel Town	0.869*	0.696*	0.903*	0.748*	0.888*							
7 Ruamahuanui Is.	0.992*	0.932*	0.974*	1.000*	0.972*	0.880*						
8 Kauraeranga Valley	0.833*	0.829*	0.884*	0.844*	0.797*	0.730*	0.713*					
9 Taupo	0.982	0.955*	0.975*	1.000*	0.981*	0.931*	1.000*	0.905*				
10 Lake Waikaremoana	0.856	0.910*	0.922*	0.936*	0.939*	0.854*	0.955*	0.828*	0.904			
11 Taranaki	0.938	0.943*	0.961*	0.970*	0.961*	0.917*	0.981*	0.897*	0.922	0.905*		

12 Palmerston North	0.458*	0.651*	0.609*	0.565*	0.608*	0.573*	0.583*	0.535*	0.424*	0.471*	0.531*	
13 Wellington	0.671*	0.787*	0.752*	0.750*	0.786*	0.759*	0.794*	0.738*	0.634*	0.661*	0.662*	0.490*

Table 3.4. Analysis of molecular variance (AMOVA) using ARLEQUIN (Excoffier *et al.* 2005) among ground weta from 13 populations in NI, New Zealand.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	*p*
Among populations	12	1.691	0.02039 Va	79.42	< 0.001
Within populations	75	0.396	0.00528 Vb	20.58	
Total	87	2.087	0.02567		
Fixation Index		Φ_{ST}: 0.79418			

3.3.3 Isolation-by-distance

Genetic differentiation and geographical distance were not significantly correlated at any of the three geographical scales (Fig. 3.12), indicating that genetic distances among populations varied independently of the geographic proximity between populations.

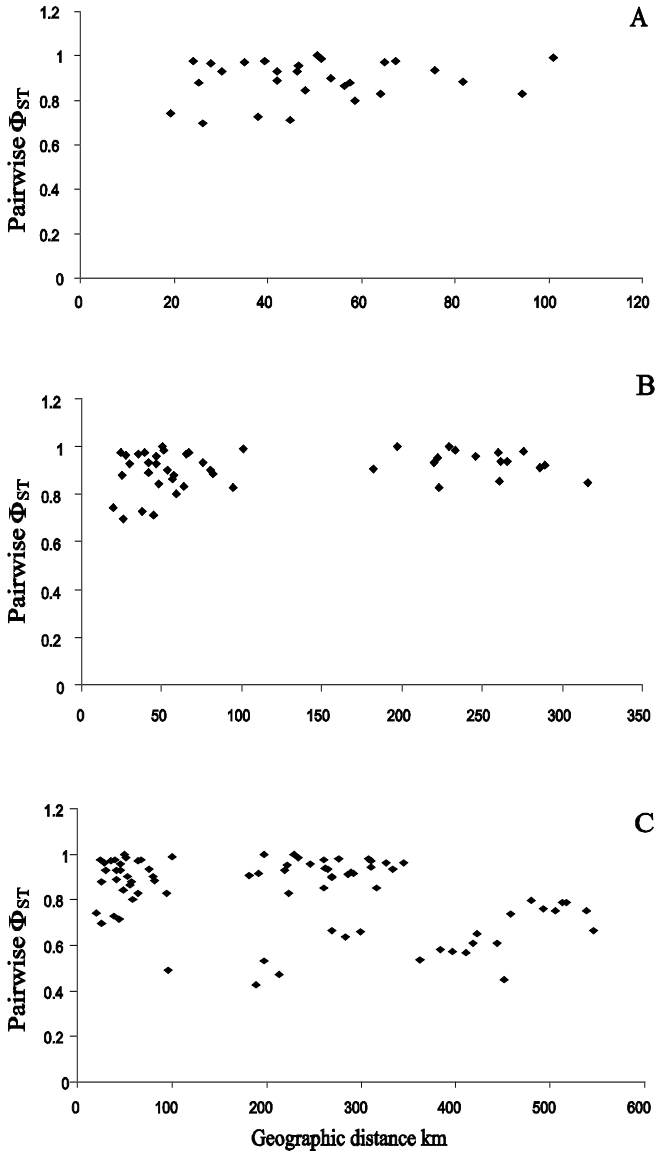

Figure 3.12. Isolation-by-distance plots for (A) Little Barrier Island, the Coromandel Peninsula and surrounding islands - Z score = 13648, R^2 = 0.137551, r = -0.370878, p = > 0.05, (B) the above plus Taupo and Lake Waikaremoana - Z score = 5040, R^2 = 0.062945, r = 0.250889, p = > 0.05, and (C) the above plus Taranaki, Palmerston North and Wellington - Z score = 1269, R^2 = 0.036622, r = 0.191369, p = > 0.05 (see Fig. 3.8).

3.3.4 Genetic distances and molecular clock

For the long dataset containing the outgroup species, uncorrected *p* genetic distances had a maximum of 16.4%. Most (96%) of pairwise distances between species were greater than 0.105 (10.5%) (Fig. 3.13); nine pairwise distances between *H. bilobatus* and *H.* "promontorius", and two between *H.* "horomaka" and *H.* "onokis", were less than 0.045 (4.5%). Substantial genetic structuring was evident within *H. pallitarsis*, with the highest uncorrected pairwise genetic distance (8.9%) found between weta from Taupo (GW200) and Wanganui (GW190) (see Appendix G). The pairwise genetic distance within clade I reached 5.9% (mean = 3.8%), clade II reached 6.3% (mean = 3.7%), and clade III reached 5.3% (mean = 2.9%). Between clades I and II the highest genetic distance was 8.9% (mean = 7.0%), clades I and III got to 8.2% (mean = 7.3%) and between clades II and III the greatest genetic distance was 8.2% (mean = 7.0%) (Appendix G). Estimates of genetic differentiation using the more complex nucleotide substitution model (TVM + I + G) were higher than the observed pairwise distances. For example, the TVM + I + G distance within *H. pallitarsis* reached 17.0% (mean = 8.0%) compared to the observed distance of 8.9% (mean = 5.3%).

Using molecular clock calibration rates of 1.4 – 2.6% sequence divergence per million years, the three clades within *H. pallitarsis* appear to have diverged during the Pliocene (~2.0 – 6.3 Ma). Both northern clades (I and II) diverged from the Wellington clade (III) approximately 2.0 - 5.9 Ma and have been separated from each other for about the same amount of time. The two populations on either side of the Manawatu River appear to have diverged between 2.5 and 5.5 Ma. In the Coromandel, the populations on islands east of the peninsula diverged from the Coromandel Peninsula populations during the mid Pleistocene to mid Pliocene (Cuvier Island: 1.46 – 4.86 My; Middle Island: 0.66 – 3.82 My; Ruamahuanui Island: 0.42 – 3.14 My).

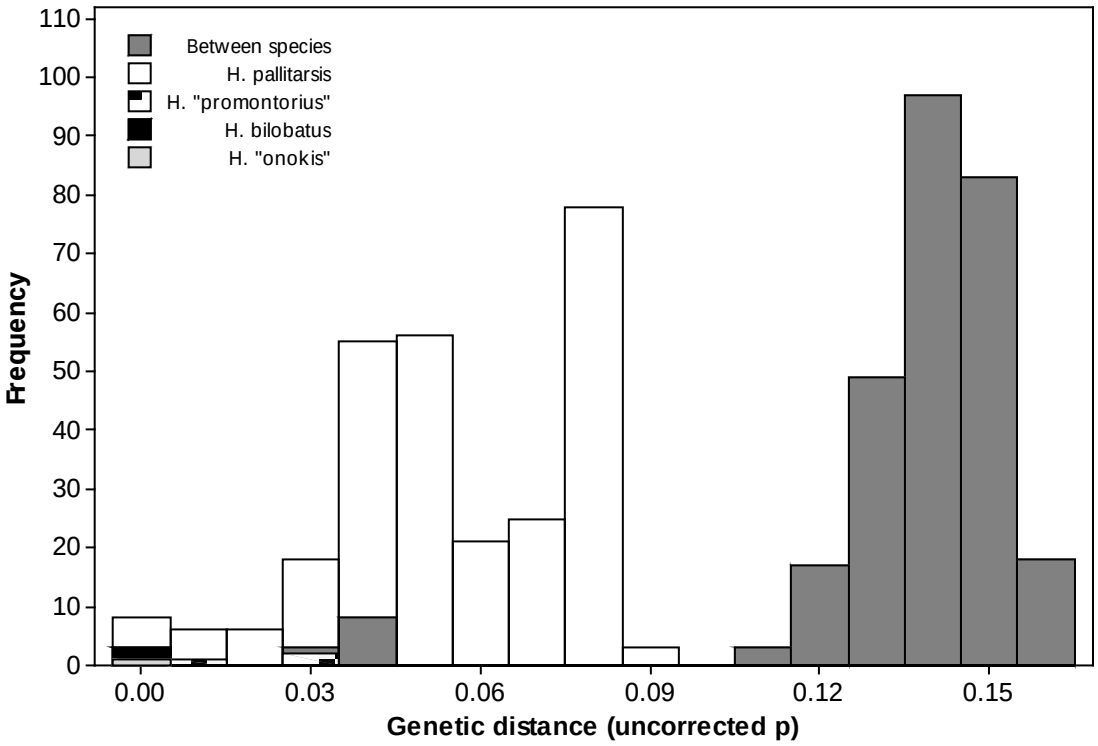

Figure 3.13. Frequency distribution of pairwise uncorrected *p* genetic distances from COI mtDNA sequences from four *Hemiandrus* species, showing pairwise distances between (dark grey bars) and within species.

3.4 Discussion

3.4.1 Phylogenetics

Hemiandrus pallitarsis is found in the North Island of New Zealand, from Wellington to Auckland (and is sympatric with several long ovipositor species and one short ovipositor species, *H. bilobatus* (Johns, 2001)). Phylogenetic analysis supports the monophyly of *H. pallitarsis* sampled through this range and on offshore islands, with respect to five of the six short ovipositor species in New Zealand. Phylogenetic structure among the short ovipositor species confirmed the sister relationship predicted by Johns (2001) between *H.* "promontorius" and *H. bilobatus*, and also found these two species form a clade sister to *H.* "vicinus" from the Marlborough Sounds. This study also found *H.* "horomaka" from Banks Peninsula is sister to *H.* "onokis" from the Canterbury region, but was unable to resolve the relationship between *H. pallitarsis* and these other short ovipositor species.

3.4.2 Genetic distances

In a global context, the high level of genetic divergence found within *H. pallitarsis* at this locus (up to 8.9%) is more typical of interspecific divergence in insects studied in the Northern Hemisphere (Funk *et al.*, 1995; Funk, 1999; Langor & Sperling, 1997), but is consistent with other studies of New Zealand invertebrates. Within the weta family Anostostomatidae, COI intraspecific uncorrected genetic distances have been reported up to 9.5% in Auckland tree weta *Hemideina thoracica* (Morgan-Richards *et al.*, 2001), up to 7.6% in alpine scree weta *Deinacrida connectens* (Trewick *et al.*, 2000) and between 5.3% and 7.1% for several other tree and giant weta species (Trewick & Morgan-Richards, 2005). The highest uncorrected distance among populations of *H. pallitarsis* was 8.9% which, although it falls within the range found in other Anostostomatids, is considerably smaller than what has been found in a New Zealand mite havestman, *Aoraki denticulata* (e.g. 14.4%, Boyer *et al.*, 2007). These large genetic distances, in conjunction with the molecular phylogeny which shows branch

lengths within *H. pallitarsis* are longer than branches between the other five short ovipositor species, may suggest a faster rate of molecular evolution in *H. pallitarsis* compared to other short ovipositor ground weta (Boyer *et al.*, 2007).

3.4.3 Population structure and gene flow

This study of mtDNA variation found that populations of *H. pallitarsis* were highly spatially structured. Despite the large genetic distances found within some populations (e.g. Wellington - 5.3%, Palmerston North – 7.7%), AMOVA indicated that the majority of genetic diversity was partitioned among populations rather than within populations (Table 3.4). High haplotype diversity was found in *H. pallitarsis*, with every location possessing a unique haplotype and in several locations every individual sequenced yielded a unique haplotype (Table 3.2). There was absolutely no haplotype sharing between localities, even those separated by less than 20 kms. In addition, low nucleotide diversity ($\pi = 0.0086$) and high Φ_{ST} values were found. All these results show substantial population structuring and suggest little recent gene flow between populations of *H. pallitarsis*. This may imply that ground weta, in particular female weta due to the inheritance of mitochondrial DNA, do not disperse far and may also have very small home ranges. Although there is a paucity of information on the dispersal ability and general ecology of ground weta, a recent study looked at insect colonization of progressively isolated peat bog plants (*Sporadanthus ferrugineus*) from a source population (Watts & Didham, 2006). They found *H. pallitarsis* at the source population but not in plants 30 m or further from the source population. These results support the idea that *H. pallitarsis* individuals do not travel far, and are unlikely to colonize isolated habitats. It is difficult to imagine, however, that habitat fragmentation has restricted dispersal and that weta are completely confined to isolated patches of vegetation for two reasons: firstly, *H. pallitarsis* occupies in a wide range of habitat types from mown lawn in urban gardens, to dense patches of Wandering Jew (*Tradescantia fluminensis*), to native forest (E. Chappell, pers. Obs.), and seems not to be reliant on native vegetation; secondly, juvenile ground weta are often observed "pinging" or jumping on the ground at night, often along open walking tracks, and can cover many metres when disturbed. Therefore, it seems likely that *H. pallitarsis* are capable of travelling across areas of open habitat, however further research is required

to fully understand the dispersal abilities of all stages of the life cycle. The finding of high haplotype diversity, coupled with low nucleotide diversity, suggests each population was formed by an independent founder event and over time genetic drift has resulted in each population becoming genetically distinct (Holland & Cowie, 2007).

3.4.4 Phylogeography

Based on the geophysical history and patterns of endemicity and biodiversity in NI, several predictions were made regarding the phylogeography of *H. pallitarsis*: firstly, that population expansion would occur in a 'north to south' direction with the lowest genetic diversity occurring in lower NI; secondly, range expansion would occur in a 'south to north' direction; or thirdly, any effect of Pliocene impacts on the phylogeographic signal would be masked due to more recent geophysical events. Population genetic analysis revealed Region 3 (populations from Wellington) had the highest level of genetic diversity and the northern NI region (Region 1) contained the lowest genetic diversity (Table 3.2). Bayesian analysis of the longer data set found *H. pallitarsis* from Wellington were sister to all the other individuals sequenced (Fig. 3.9). These results support the prediction of range expansion in a 'south to north' direction possibly following the pattern of land connection and uplift in the lower NI during the late Pliocene – mid Pleistocene (see Fig. 3.1). The Bayesian analysis also suggests the other short ovipositor NI species, *H. bilobatus*, may have moved north as it and *H.* "promontorius" are sister to the southern species, *H.* "vicinus".

This south to north pattern of population expansion was also found in New Zealand freshwater crayfishes (koura) which appear to have moved from north east South Island into central NI, and then from there into northern NI and south again to Wellington (Apte *et al.*, 2007). Apte *et al.*, also found a clear lineage division within the NI clade, separating northern NI koura from those found on the east and west coasts and from the lower NI. They suggest this northern lineage formed as a result of marine inundation during the Pliocene. No obvious northern lineage was found in this ground weta study, in fact, sequences from lower NI populations (Wanganui, Pohangina Valley and Palmerston North) grouped with all of the northern populations (Fig. 3.10). Additionally, it is unlikely that *H. pallitarsis* has re-colonized the northern half of NI in a stepwise manner through leading-edge re-colonization (Hewitt, 1999; Phillips, 1994),

as no effect of genetic isolation by distance was found, even at the smallest geographical scale (Fig. 3.12A-C). Instead of continuous active dispersal across the landscape where new populations are constantly established ahead of the main population (Hewitt, 1999), passive dispersal, mediated by vectors such as flooding events and possibly animals, may explain the present-day phylogeographical patterns. Although there have been no observations of passive dispersal in ground weta, eggs and early instar nymphs live in burrows in the ground and so may be amenable to passive dispersal through flooding events or land slides where the top layer of soil is displaced. As an aside, in species which exhibit low dispersal distances, phylogeographic breaks can arise as a result of genetic drift and not due to geographic barriers (Irwin, 2002).

Of course geographic barriers can play an important role in preventing gene flow, and can result in genetically divergent populations over time. Land mass in the NI of New Zealand during the Pliocene was less than that seen today and was fragmented into a series of islands (Fleming, 1979). As stated earlier, much of the lower NI seen today was below sea level and formed a 'proto-strait' which later narrowed to become the Manawatu Strait, separating land in the Wellington region from the northern half of the NI. It is tempting to suggest these 'straits' (and now the Manawatu River) have prevented gene flow between northern and southern populations – Palmerston North populations on the northern side of the Manawatu River are genetically divergent from those on the southern side of the river (Fig. 3.10). However the phylogeographical pattern found in *H. pallitarsis* is not that straightforward. Several northern NI populations (Cambridge, Taupo and Taranaki) form a clade with one of the Palmerston North populations (from the south side of the Manawatu River); indicating weta may be capable of dispersing across this geographic barrier. It is possible that this phylogeographic pattern is a result of stochastic population extinction and expansion following habitat shifts during the glacial-interglacial cycles in the Pleistocene. Alternatively, the current phylogeographical pattern may have arisen due to more recent volcanic activity in the central NI. A large area of land surrounding Lake Taupo was either covered in pyroclastic rock or ash (See Fig. 3.3A), effectively destroying the habitat and so it is unlikely weta were able to survive in this area. Therefore, it is possible that secondary contact of allopatrically differentiated populations has occurred (Morgan-Richards *et al.*, 2000).

3.4.5 Molecular clock and its caveats

Using a molecular clock calibration range of between 1.4 and 2.6% sequence divergence per million years (Knowlton & Weigt, 1998; Knowlton *et al.*, 1993), mtDNA lineages within *H. pallitarsis* apparently diverged during the Pliocene (~2 - 6.3 Ma). Several geographically proximate populations, such as the two Palmerston North sites, were highly genetically divergent. For example, the two reciprocally monophyletic clades in the Wellington region are separated by only ~7 kms, with no obvious barrier in between, but using the molecular clock calibration rate they have apparently diverged 3.8 – 1.7 Ma (mid Pliocene – early Pleistocene). Uncorrected genetic distance data suggests the island populations of *H. pallitarsis* off the east coast of the Coromandel Peninsula diverged from the mainland between 0.4 and 4.9 Ma. Considering these islands were connected to the Coromandel Peninsula at the last glacial maxima (~22,000 years ago) and, due to the cyclical pattern of sea level fluctuations during the Pleistocene, have had the opportunity for intermittent gene exchange until around 12,000 years ago, it is surprising to find such a high level of genetic divergence. Of course if this species does have a faster rate of mtDNA COI evolution, as suggested earlier, these divergence times will be overestimates, and the actual timing may be much more recent.

However, research based on pedigree mtDNA data in humans and Adélie penguins have shown extremely high mutation rates, exceeding the tradition rate of 1% per million years (Howell *et al.*, 2003; Lambert *et al.*, 2002). Contrasting substitution rates between population-level studies and phylogenetic studies led Ho *et al.* (2005), to examine the differences between short-term mutation rates and long-term substitution rates in avian and primate data. They found a reverse 'J' curve relationship between calibration age and the rate of change, that is, molecular rates decrease exponentially with increasing time. Likewise, Burridge *et al.* (2008) also found evidence for "time dependency of molecular rates" in New Zealand freshwater fishes. Overall, it appears that molecular rates vary across different evolutionary timescales and so using a rate based on phylogenetic data for a population-level study will give misleading inferences. Based on this information, it is likely that *H. pallitarsis* does not have a faster rate of mtDNA COI evolution, and that population divergence has probably occurred some time during the Pleistocene.

3.4.6 DNA barcoding and morphology

DNA barcoding is a taxonomic method aimed at reliably and cost-effectively delimiting species and detecting cryptic species (Hebert *et al.*, 2003). Usually the 5' end of the mitochondrial gene, cytochrome *c* oxidase I, has been used to determine levels of genetic diversity. Despite the initial attraction of this method, subsequent applications of DNA barcoding have found it is not universal and may be misleading in cases where ecological and morphological information is missing (Trewick, 2008). Meyer and Pauley (2005), highlight the importance of greater nucleotide diversity between species than within species. In this study pairwise genetic distances between four short ovipositor species of ground weta were greater than those found within *H. pallitarsis* (Fig. 3.13). These results, in conjunction with the morphometric analyses in Chapter 1, support the current recognized taxonomy for *H. pallitarsis.* Several recommendations have been put forward, however, to help solve issues regarding the use of DNA characters for species identification and delimitation, including the use of multiple characters, such as morphology and behaviour, and sequencing longer DNA fragments, around 1000 bp (with modern sequencing methods fragments of this size can usually be sequenced using a single set of primers) (Roe & Sperling, 2007).

Overall this study found substantial genetic structuring within *H. pallitarsis*, with large genetic distances, high haplotype diversity, low nucleotide diversity and no haplotype sharing among populations. Analysis of mtDNA COI data revealed phylogeographic patterns consistent with a south to north pattern of movement, possibly related to land uplift in the lower North Island during the Pliocene. Phylogeographic patterns among populations of *H. pallitarsis* may have also been affected by Pleistocene climate change and volcanic activity. Genetic analyses, in conjunction with morphometric analyses give no indication of a species complex within *H. pallitarsis,* but suggest it is a highly genetically variable species.

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Chapter 4: Behavioural Drumming

Final stage of copulation in *Hemiandrus pallitarsis*, with male (below) delivering the spermatophylax to the mid abdominal region of the female (above).

Chapter 4: Vibratory drumming variation in three populations of *Hemiandrus pallitarsis*

4.1 Introduction

4.1.1 Vibrational communication

Insects use a range of mechanisms for communication, including vibration (and tremulation), percussion, stridulation, click mechanisms and air expulsion (Ewing, 1989), and in some cases a complex combination of these mechanisms is employed (Claridge, 2006). "Silent songs", produced through substrate vibrations, are a widespread method of communication among insects (Cocroft & Rodriguez, 2005; Henry, 1994; Virant-Doberlet & Cokl, 2004). There are different levels of vibrational communication: near-field air pulsing (Henry, 1994), where individuals in close proximity use their wings to vibrate the air (Bennet-Clark, 1971); drumming, where an insect directly vibrates a body part against the substrate (Stewart *et al.*, 1991); and tremulation, where an insect vibrates a part of its body without striking the substrate (indirect vibration) (Morris, 1980). Vibrational communication, particularly in association with mate finding and courtship, is thought to be advantageous over the use of acoustic signals because it takes away the potential problems of attracting predators (Bell, 1980; Henry, 1994; Markl, 1983), despite this Cocroft and Rodriguez (2005), list a range of predators capable of eavesdropping on insect vibratory signals (e.g. spiders, Barth, 1997; Morris *et al.*, 1994; Hemiptera, Pfannenstiel *et al.*, 1995; and parasitoid wasps, Casas *et al.*, 1998). It can also be disadvantageous due to the short transmission distance (e.g. connectivity of plants) (Henry, 1994). Substrate transmitted signals, like vibratory signals, are used in species recognition and can serve as an effective pre-mating barrier (Henry, 1993; Henry *et al.*, 1999), but are also used for sexual advertisement and portraying aspects of mate quality.

4.1.2 Species recognition signals

In the past, sexual signals such as songs, body posturing and displays, were thought to primarily function in species recognition and reproductive isolation (Darwin, 1859, 1871; Henry, 1994). For example, extensive research on bird song has found birds can discriminate between conspecific and heterospecific songs (Baker & Baker, 1990; Nelson & Marler, 1993; Ratcliffe & Grant, 1985; Searcy *et al.*, 1995). The process where songs or signals are used by organisms to discriminate conspecifics was originally described by Paterson (1985), which he termed the specific-mate recognition system (SMRS). This is where an individual produces a signal, such as a song, that another individual of the same species receives, recognizes and responds to. A communication system like this is important for successful fertilization between genetically compatible conspecifics (Wilczynski & Ryan, 1999), and in cases where species ranges overlap (i.e. sympatric species) differences in sexual communication signals can be accentuated. Seddon (2005), for example, analysed acoustic calls of 21 species of Thamnophilid antbirds in the Neotropics. For each species the author compared call structure between a close relative in sympatry and one in allopatry and found that the songs of closely related sympatric species were more divergent that those of closely related allopatric species. Traditional theory suggests greater divergence between species signals in sympatry results from increased selection to limit producing infertile hybrids (Brown & Wilson, 1956; Dobzhansky, 1951), and so courtship signals that reliably reflect species identities are favoured.

Calls used as "species recognition signals" are mutually recognized by conspecifics of each sex, and so it has been suggested that these signals should not vary greatly across a species geographical range due to stabilizing selection (Butlin, 1995; Paterson, 1985; Wilczynski & Ryan, 1999). In other words, as long as members of a species occupy their normal habitat, there should be little geographic variation in specific-mate recognition characters. In spite of this, species recognition signals can substantially vary across populations (Prohl *et al.*, 2007; Ryan *et al.*, 1996a; Ryan & Wilczynski, 1991; Ryan *et al.*, 1996b; Tregenza *et al.*, 2000), suggesting only certain

aspects of these signals may be used for species recognition. As an example, Wilczynski *et al.*, (1995), manipulated the advertisement call of the Neotropical frog (*Physalaemus pustulosus*) and found signal frequencies anywhere between 900 – 560 Hz, followed 50 ms later by a signal between 640 – 500 Hz was all that was necessary for species recognition, much of the natural call variation was associated with intraspecific mate choice.

4.1.3 Factors shaping the evolution of sexual signals

There are a myriad of factors that can shape the evolution of sexual communication systems and that can account for geographic variation. These factors can be divided into two categories: ecological and social factors.

Ecological factors:

Habitat structure and composition, can directly affect transmission of calls and signals (e.g. Zimmerman, 1983; Slabbekoorn & Smith, 2002; Tubaro & Lijtmaer, 2006), and in the case of vibrational signals, the distance of transmission is limited to the characteristics of the plant and connectivity to other plants (Cokl & Virant-Doberlet, 2003). Environmental factors such as altitude and temperature can also affect advertisement calls (Narins & Smith, 1986). It has long been recognized that temperature affects the calling song of many Orthopteran groups, e.g. Gryllidae (Brooks, 1882; Dolbear, 1897; Prestwich & Walker, 1981; Toms *et al.*, 1993), Tettigoniidae (Frings & Frings, 1957; Gwynne & Bailey, 1988; Walker *et al.*, 1973), and Acrididae (Skovmand & Pedersen, 1983; von Helverson & von Helverson, 1994). Generally, as temperature increases the pulse rate of the song (pulses per second) increases linearly. Such an effect is not surprising when you take into account the fact that these insect groups use muscle and the nervous system to produce sound, and hence Sanborn (2006) emphasized the importance of measuring body temperature, or ambient air temperature when recording calling insects. Interestingly, 'silent' songs are also linearly affected by temperature – increased temperature results in an increased drumming rate (drums per second) (Weissman, 2001).

Temperature can also indirectly affect communication systems through selection on body size. Ectotherms generally tend to be smaller in colder temperatures (Mousseau, 1997; Park, 1949; Partridge & Coyne, 1997; Van Voorhies, 1997), and it has been extensively shown in insects that larger males tend to have larger sound producing structures that produce lower frequency calls (Bennet-Clark, 1998; Gerhardt & Huber, 2002; Ryan & Brenowitz, 1985), which can travel over longer distances (Bradbury & Vehrencamp, 1998).

Social factors:

Sexual selection and character displacement can also result in intraspecific population variation in sexual signals. The process of sexual selection can be loosely defined as competition for fertilization opportunities with the opposite sex. Competition is usually in the form of male-male competition but cryptic female choice can also result in sperm competition (Eberhard, 1996). Character displacement is where historically separated species now have overlapping ranges but which produce infertile hybrids if mated (Butlin, 1987). Sexual signals can be species specific, but mate quality information can also be included in the calls. For example, many studies have examined the effect of female choice on conspecific male signals and found females are attracted to some signals over others (Orci, 2007; Parri *et al.*, 2002; Ryan & Keddy-Hector, 1992; Tokarz, 1995; Wilczynski *et al.*, 1995; Wilczynski & Ryan, 1999). Moreover, in many cases sexual selection can act on body size with females preferring larger bodied males (Bateman *et al.*, 2001; Brown *et al.*, 1996; De Luca & Morris, 1998; Gwynne, 1982; Simmons, 1992), which as mentioned earlier, can produce lower frequency calls that can travel further.

Where there are no barriers to migration and there is gene flow between geographically close populations, variation in sexually selected signals among populations can follow a gradient (cline) across a landscape (Lande, 1982). For example, Ryan *et al.*, (1996) analysed patterns of advertisement call variation in the túngara frog (*Physalaemus pustulosus*) and found that for some of the call variables there was a clinal pattern of variation. However, they found that the majority of the call variables did not show clinal variation and instead varied between two major allozyme groups. Therefore, sexual selection can also generate a non-linear relationship between calling signal and

geographic distance (i.e. no isolation-by-distance effect). In extreme cases, variation in sexually selected signals among populations can lead to speciation through population divergence (West-Eberhard, 1983).

4.1.4 The New Zealand ground weta: Hemiandrus pallitarsis

In this chapter I describe the variation in male vibratory signals between three populations of *Hemiandrus pallitarsis.* Previous research has found evidence that vibratory signals are used for mate attraction in *Hemiandrus* (formerly *Zealandosandrus*) *subantarcticus* (Butts, 1983) and *H.* (formerly *Z.*) *maculifrons* (Cary, 1981). More recently, Gwynne (2004), analysed video recordings of vibrational drumming from four species of ground weta in New Zealand: seven *H. pallitarsis* weta from two locations in the Manawatu area, three *H.* "onokis" and three *H.* "vicinus" weta, and a single *H.* "promontorius" individual. All recordings were made inside a garden hut in Palmerston North. He found the length of the drumming bout and the number of pulses in each bout was species specific. He also made detailed observations of the vibratory drumming behaviour of *H. pallitarsis*. Gwynne observed adult male weta drumming their abdomens on the substrate, usually a plant surface such as a leaf or stem, and females drumming in apparent response to the male signal. The female drumming pattern appears to differ from that of the male; this however, is based on a recording from a single female. Hence, the complex sexual signalling behaviour of *H. pallitarsis* makes this a useful and interesting species in which to investigate intraspecific population variation in vibratory calls. Gwynne's 2004 study highlighted the species specific nature of vibratory drumming behaviour in several species of *Hemiandrus*, but did not touch on the level of intraspecific variation or, intra- and inter- male variation in these vibrational signals. No other studies have examined in detail the vibrational mating behaviour of New Zealand weta, despite its possible importance in reproductive isolation and speciation.

4.1.5 Hypotheses

Morphometric and molecular analysis of *H. pallitarsis* populations in previous chapters lead to two predictions in the pattern of drumming behaviour expected in this species: my first prediction based on morphometric data (Chapter 2) and geographical proximity is that populations on either side of the Manawatu River would exhibit more similar drumming behaviour patterns compared to Coromandel Peninsula weta (Fig. 4.1a); alternatively, based on genetic data (Chapter 3) I predict weta on the north side of the Manawatu River would exhibit more similar drumming behaviour patterns to Coromandel Peninsula weta than they would to weta south of the river due to genetic similarities (Fig. 4.1b).

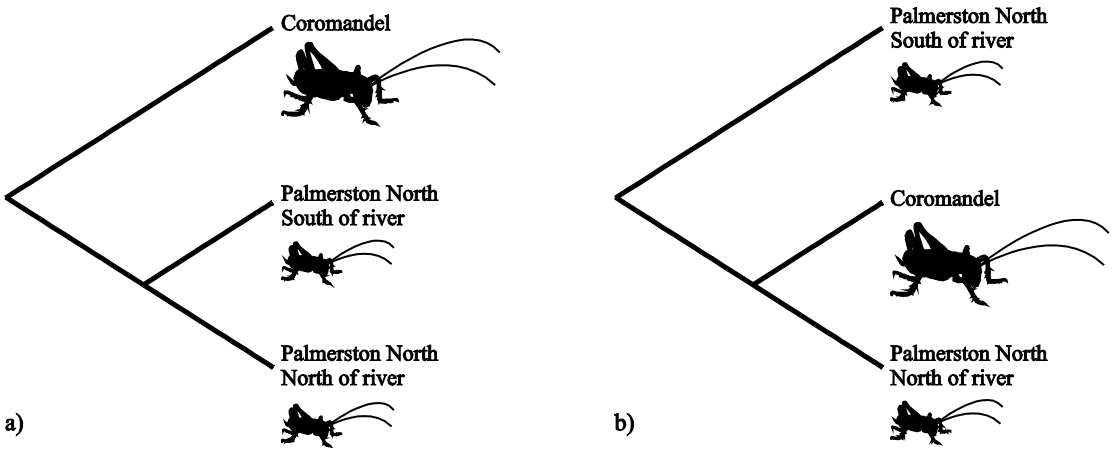


Figure 4.1. Trees illustrate predicted patterns of drumming behaviour in *Hemiandrus pallitarsis*: a) Morphometric/geographic hypothesis – smaller weta from geographically proximate populations will show behavioural similarities; b) Genetic hypothesis – genetically similar populations (Coromandel and Palmerston North, north of the river) will have drumming patterns more similar to each other than to Palmerston North, south of the river.

4.2 Methods

4.2.1 Study populations

Three populations of *H. pallitarsis* were used for this study (Fig. 4.2): Coromandel Town (36°44´ S, 175°30´ E); a single location on the northern side of the Manawatu River - Manawatu-North (40°22´ S, 175°35´ E); and a single location at Massey University, Palmerston North, on the southern side of the Manawatu River - Manawatu-South (40°23´ S, 175°36´ E). The two most separated locations, Coromandel and Manawatu-South, are approximately 400 km apart, while Manawatu-North and Manawatu-South are less than 2 km apart but are separated by the Manawatu River. All individuals at each location were collected within approximately a 100 m^2 area.

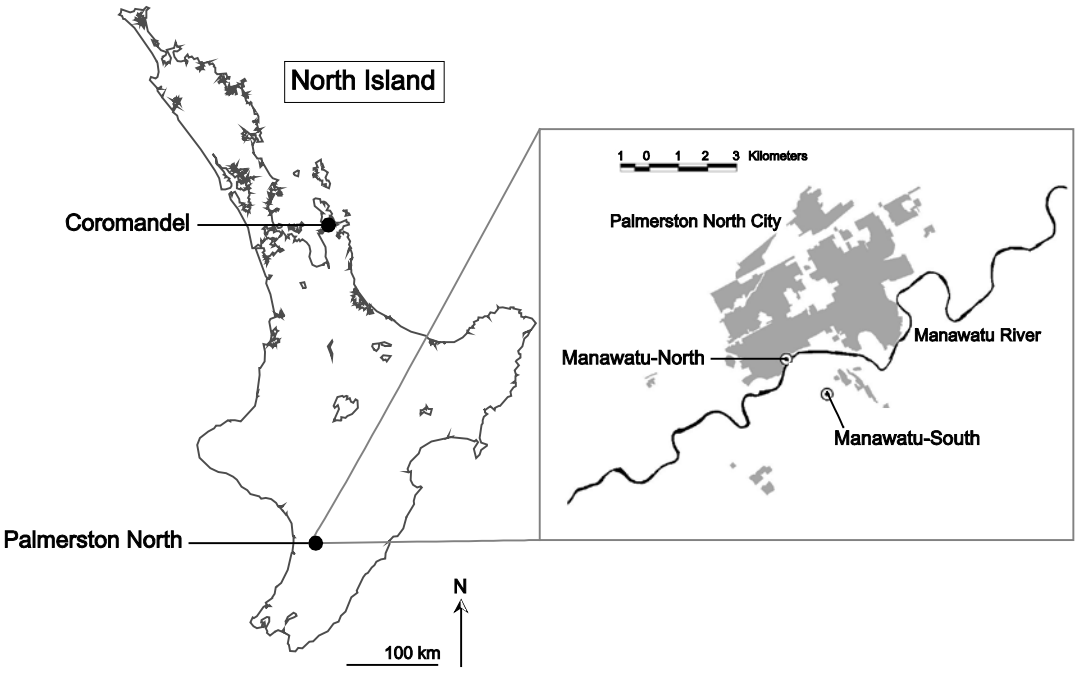


Figure 4.2. Sampling and recording locations of *Hemiandrus pallitarsis,* in North Island, New Zealand.

4.2.2 Recording techniques and drumming measurements

Adult male *H. pallitarsis* were recorded from mid February through to early March, 2008. A pilot study showed that weta did not drum consistently after several hours or over an extended period (days to weeks) in captivity. Therefore, drumming behaviour was recorded in the field within 10 metres of where the weta was caught and within minutes of capture. Weta were hand collected at each field site after dark (from approximately 9:00pm - 12:00am), and placed in individual plastic containers (4.4 x 5.8 mm) with holes in the lid for ventilation. The lid was removed and the container inverted onto the recording device so that the weta was left sitting directly on the recording device but could not move beyond the confines of the container (see Fig. 4.3). The recording device consisted of a Piezo film (3/8 1″) contact pickup (obtained from www.windworld.com/index.htm) – a device which detects vibratory movement – taped down to a small portable surface and connected to an Olympus model VN-2100PC digital voice recorder (Fig. 4.3). Each weta was left on the recording device for approximately 30 minutes (17 - 209 minutes), after which time the weta was marked on the pronotum with white correction fluid and released back to where it was found - this prevented the same individuals from being collected twice, and did not appear to be harmful as males with correction fluid were seen on following nights mating. Because temperature has been found to linearly affect the calling-song drumming rate in other Orthopterans (e.g. Jerusalem crickets, Weissman, 2001), the ambient air temperature was measured using a digital thermometer placed at the base of the recording device at the beginning of each recording session.

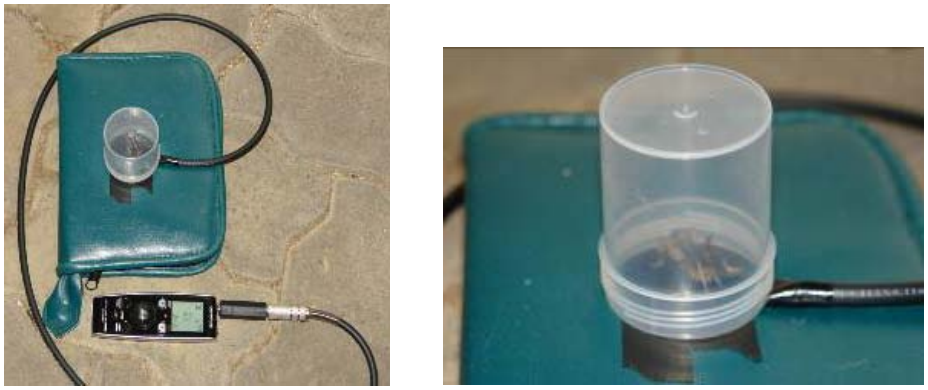


Figure 4.3. The recording setup showing a male weta inside a plastic container sitting on top of the Piezo film (beneath black electrical tape). Photos – E.M. Chappell.

Recordings were analysed using the sound analysis software, Audacity 1.2.6 (http://audacity.sourceforge.net). Each drumming bout was amplified so each pulse within the bout, and each oscillation within a pulse could be clearly seen (Fig. 4.4). The following drumming parameters were measured directly from the oscilloscope: 1) Total drumming bout duration in seconds (Bout length), 2) Number of pulses within a drumming bout (Pulses), 3) Mean number of oscillations within one pulse (Oscillations), 4) Inter-pulse interval (seconds) (Inter-pulse), and (5) the number of pulses per second (Pulse rate) (Fig. 4.4).

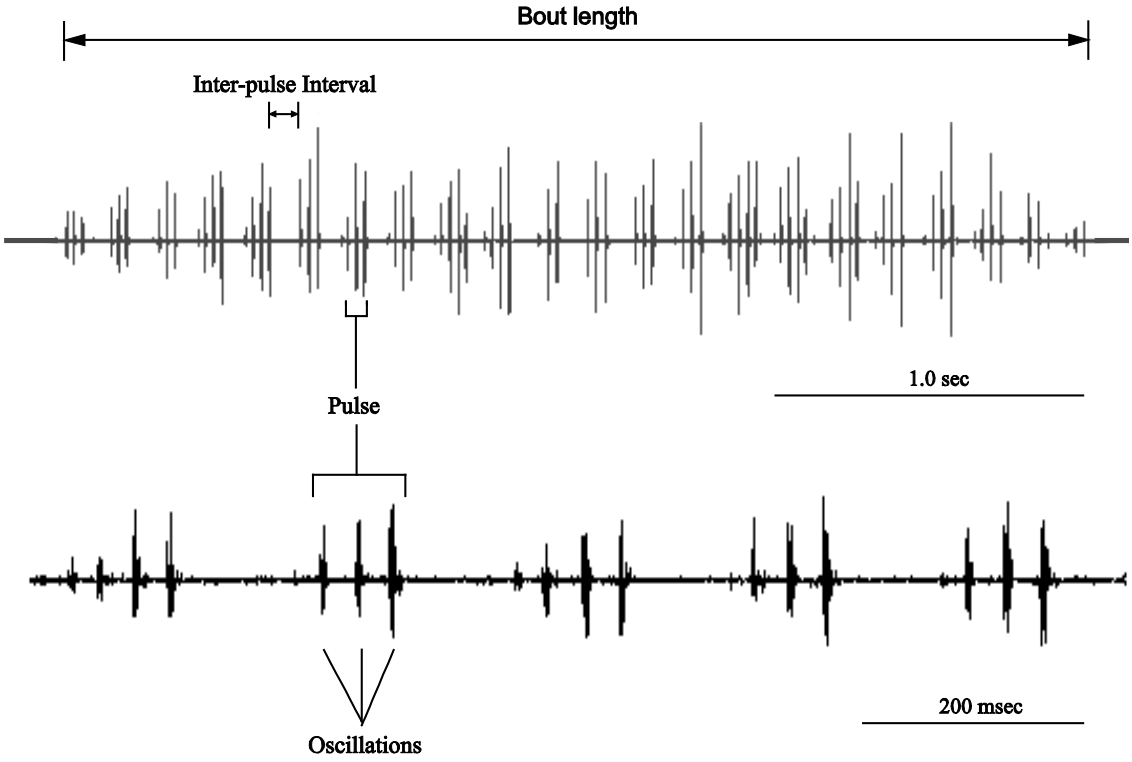


Figure 4.4. Oscillograph of a single drumming bout produced by a *H. pallitarsis* male showing measurements taken: top trace shows bout length, inter-pulse interval and number of pulses; bottom trace shows five pulses from within the drumming bout, indicating the number of oscillations within each pulse.

4.2.3 Statistical analyses

SPSS software package (SPSS ver. 13.0, SPSS, Inc., Chicago, IL) was used to obtain descriptive statistics (mean and standard deviation) of drumming bouts for each individual weta in which more than one drumming bout was recorded, and for each population. Pearson *r* correlation coefficients were used to evaluate the relationship between each of the drumming parameters measured. MLwiN version 2.02 statistical software (Rasbash *et al.*, 2005) was employed for testing intra- and inter-male variation and population variation. To test the assumption of normality, standardized residuals for each drumming parameter were plotted against the predicted values and the normal score. Each plot was examined for evidence of structure in the form of outliers, curved relationships or heteroscedasticity. In all cases residual plots revealed normally distributed data. A hierarchical model, with observations within specimens, was used to test whether drumming parameters differ significantly between the three populations sampled: Coromandel, Manawatu-North and Manawatu-South. For each drumming parameter (i.e. bout length, pulses), a forward selection method was employed whereby the starting model included only a constant and a population level term, then the effect of temperature was added. Two times log likelihood score, a measure of deviance, was used to determine whether adding the effect of temperature improved the model (shown as a decrease in the log likelihood score). To test whether any decline was significant ($p \leq 0.05$), a Chi-square table of critical values was used with degrees of freedom equal to the change in the number of parameters.

Initial model:

$$Y_{ij} = \beta_{0i} + \beta_{1j} + \beta_{2j}$$

Where: Y_{ij} = each drumming parameter (e.g. bout length, pulses, etc.)

i = individual level

j = observations within an individual level

β_{0i} = reference population mean + an error value of mean observations within an individual

β_{1j} = difference in mean value between population 1 and reference population

β_{2j} = difference in mean value between population 2 and reference population

Second model:

$$Y_{ij} = \beta_{0i} + \beta_{1j} + \beta_{2j} + \beta_{3temp}$$

Where: β_{3temp} = mean temperature – population mean

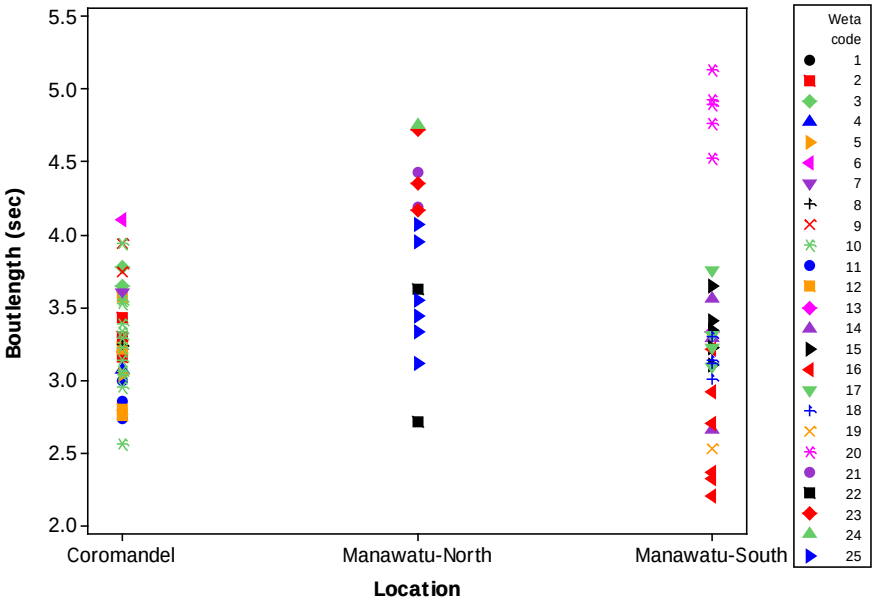
Parameters in the model were deemed significant if the sum of two times the standard error is less than the parameter estimate. The same method was employed to determine the significance of within individual variation. Significant dummy variables for the populations were taken as evidence of differences between the populations.

4.3 Results

In total, 78 drumming bouts were recorded from 25 male weta. Of these, 9 weta drummed once while 16 weta drummed between 2 and 12 times whilst on the recording device (mean = 4.312, SD = 2.522). The number of individual weta recorded at each site (*N*) varied as did the temperature range: Coromandel, *N* = 12, 17.5 - 20.3°C; Manawatu-North, *N* = 5, 13.9 - 16.8°C; and Manawatu-South, *N* = 8, 13.9 - 18.8°C.

Bout length was significantly correlated with the number of pulses ($r = 0.620$, $p = 0.001$), inter-pulse interval ($r = 0.594$, $p < 0.01$) and the pulse rate ($r = -0.703$, $p < 0.001$). The pulse rate was also significantly correlated with the inter-pulse interval ($r = -0.886$, $p < 0.001$).

In all cases, there was as much variation within individuals (i.e. at the observations level) as there was between individuals in a population after adjusting for temperature. For example, the raw data on bout length within the Coromandel population ranged from 4.1 – 2.6 s, and the greatest variation in bout length within an individual was 3.9 – 2.6 s (Fig. 4.5). For four of the five drumming components measured, adding the effect of temperature to the model significantly decreased the log likelihood score (Chi-square critical value > 5.99, $p = 0.05$). The model for the number of oscillations was not improved by adding the temperature effect and so only the initial model was used to compare population means.


Boutlength (sec)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Coromandel
Manawatu-North
Manawatu-South
Location
Weta code
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
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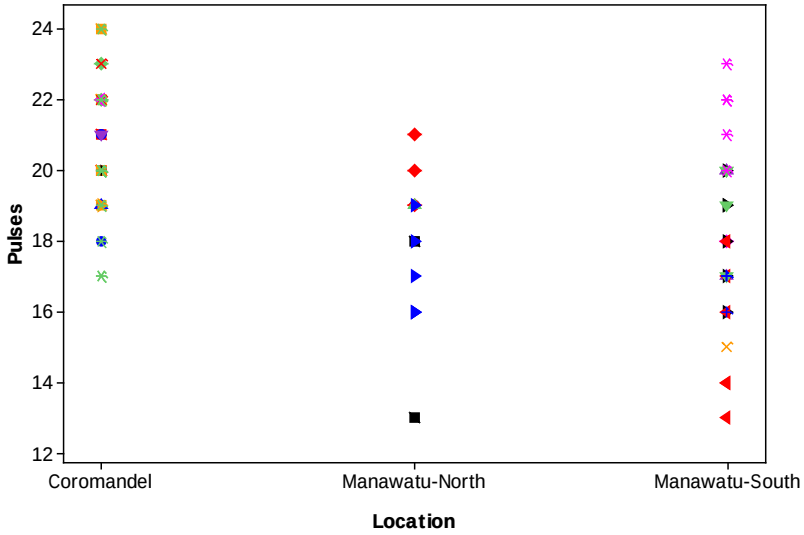
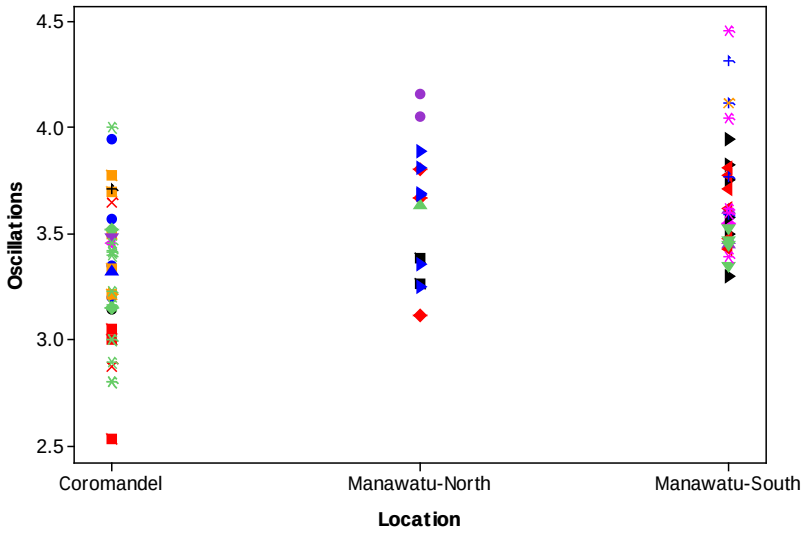

Pulses
24
22
20
18
16
14
12
Coromandel
Manawatu-North
Manawatu-South
Location


Oscillations
4.5
4.0
3.5
3.0
2.5
Coromandel
Manawatu-North
Manawatu-South
Location

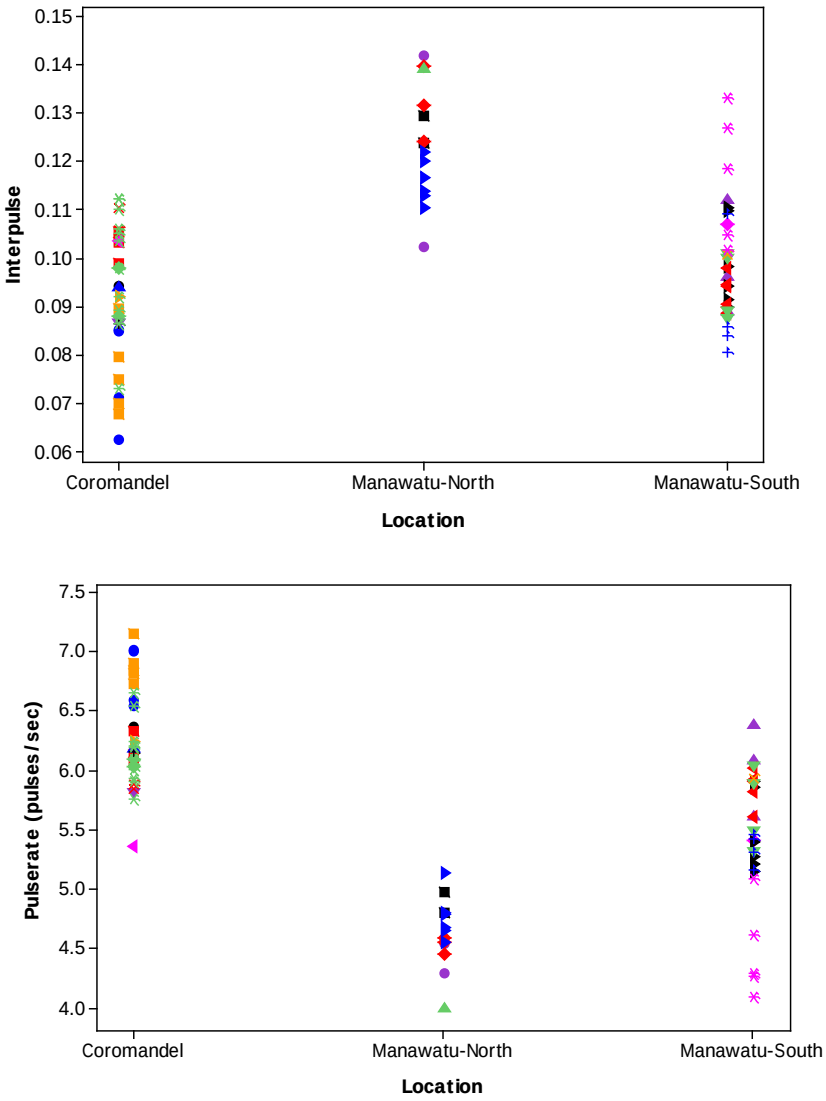


Figure 4.5. Scatter plots using the raw data not adjusted for temperature, for each drumming component measured at each of the three populations. Symbols indicate individual weta; identical symbols indicate multiple drumming bouts recorded for a single individual.

All of the drumming components revealed significant differences between Coromandel and Manawatu-North, and all components except for the inter-pulse interval showed significant differences between Coromandel and Manawatu-South (Table 4.1). Three out of the five drumming components measured revealed no significant difference between the two Manawatu populations. Drumming bouts in Coromandel were longer, contained more pulses, and subsequently had higher pulse rates than either of the two Manawatu populations, but had fewer oscillations in each pulse and a shorter inter-pulse interval (Table 4.1). As the air temperature increased, the drumming bouts got shorter,

the number of pulses and oscillations decreased, and the inter-pulse interval also decreased. However, the shorter bout lengths and fewer pulses with increased temperature resulted in a faster pulse rate with increasing temperature (Table 4.1).

Table 4.1. Mean values (±SE) for the drumming bout components of male ground weta *H. pallitarsis* from three different populations. Differences between populations and changes in drumming measurement with temperature shown on the right. * $p \leq 0.05$; ns $p > 0.05$.

Drumming component	Mean (SE)			Population comparisons (*p* values)			Temperature increase by 1°C
	Coromandel (C)	Manawatu-North (MN)	Manawatu-South (MS)	C vs. MN	C vs. MS	MN vs. MS	
Bout length (sec)	3.29 (0.087)	2.67 (0.260)	2.69 (0.155)	*	*	ns	-0.278
Pulses	20.56 (0.346)	15.27 (1.037)	16.62 (0.619)	*	*	ns	-0.591
Oscillations	3.30 (0.054)	3.62 (0.084)	3.69 (0.057)	*	*	ns	-
Inter-pulse (sec)	0.09 (0.002)	0.11 (0.006)	0.09 (0.004)	*	ns	*	-0.003
Pulse rate (pulses/sec)	6.29 (0.068)	5.55 (0.204)	5.98 (0.122)	*	*	*	0.211

4.4 Discussion

This study was the first to examine vibratory drumming patterns within a species of ground weta, *Hemiandrus pallitarsis*. Based on the degree of morphometric and genetic variation observed among populations of *H. pallitarsis*, two predictions were formulated: 1) smaller weta from the two geographically proximate Palmerston North populations would have more similar drumming patterns when compared to the larger weta from Coromandel (see Chapter 2); or 2) drumming patterns between Coromandel and populations north of the Manawatu River would have more similar drumming patterns due to genetic similarities (see Chapter 3). The pattern of population divergence followed that predicted by the morphometric/geographic proximity hypothesis (Fig. 4.6). Larger male weta from Coromandel had significantly longer drumming bouts with higher pulse rates than the smaller weta in both the Palmerston North populations. Three of five drumming components found no significant difference between the two geographically proximate Palmerston North populations. Similarity in drumming behaviour between the two Palmerston North populations contrasts with the genetic results found in Chapter 3 – Coromandel Peninsula and populations on the northern side of the Manawatu River formed a clade that was sister to populations on the southern side of the river (see Chapter 3, Fig. 3.10). Analysis of genetic data also found no evidence for genetic isolation by distance (i.e. clinal variation) among all populations sampled across the North Island (see Chapter 3, Fig. 3.12). In contrast, the pattern of vibrational drumming seemingly follows a cline, at least across part of the distributional range of *H. pallitarsis* (although only three populations were sampled in this study). Accordingly, the observed patterns of gene flow between the Palmerston North and Coromandel populations suggest other

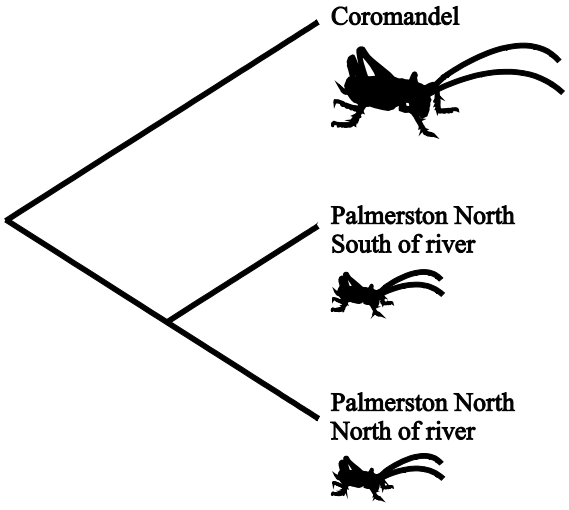


Figure 4.6. Morphometric/geographic hypothesis. - smaller weta from geographically proximate populations show more similar drumming patterns.

factors, such as ecological or social factors, may have contributed to the geographical differences in drumming behaviour in *H. pallitarsis*.

These results show that there is significant intraspecific variation in *H. pallitarsis* drumming calls, a pattern that disagrees with the expected consistency in specific-mate recognition characters across populations due to stabilizing selection (Butlin, 1995; Ferreira & Ferguson, 2002; Paterson, 1985). Finding intraspecific variation in communication signals is consistent with previous research examining population variation in insect songs. For example, Tregenza *et al.* (2000), found significant differences in the calling song of meadow grasshoppers (*Chorthippus parallelus*) between 8 populations across Europe and the United Kingdom. Gwynne (2004), first examined the vibratory drumming behaviour of ground weta, and found that the length of the drumming bout and mean number of pulses within a drumming bout varied between three species of short ovipositor ground weta. Specifically, *H. pallitarsis* had longer drumming bouts (4 – 6 sec) and more pulses per bout (18 - 21) than either *H.* "onokis" (2 – 3.1 s, and 8.7 – 12.5 pulses) or *H.* "vicinus" (1.1 – 1.8 s, and 7 – 8.6 pulses). Data from the present study suggest these characters also vary among populations within a species, and although the mean number of pulses in each population falls within the range found by Gwynne (15.3, 16.6 and 20.6), the mean duration of the drumming bouts do not (2.7, 2.7 and 3.3). It is possible then, that weta only use certain aspects of vibratory signals for species recognition (e.g. the number of pulses within a bout), and instead use a combination of mechanisms, such as pheromones, for species recognition. For example, Gwynne (2004) identified males depositing small brown droplets onto the surface of leaves where mating took place and suggests pheromones may also be involved in mate attraction. Explanations for population variation in vibratory drumming signals include social factors, such as sexual selection through female preference, or ecological factors, such as habitat structure and composition, or climate differences.

Firstly, population variation as a result of sexual selection can be generated by a bias among females for particular male signal characteristics related to mate quality (Kotiaho *et al.*, 1996; Orci, 2007; Ryan & Keddy-Hector, 1992; Tokarz, 1995; Wilczynski *et al.*, 1995; Wilczynski & Ryan, 1999). In a study examining female preference for male wolf spider (*Hygrolycosa rubrofasciata*) substrate drumming using manipulated playback signals, female wolf spiders preferred drumming bouts of longer

duration (Parri *et al.*, 2002). They suggested the length of the drumming bout may be indicative of mate quality in these spiders.

Examining levels of intra- and inter-male variation may address the question of whether *H. pallitarsis* males portray aspects of mate quality in their drumming calls. For all drumming components measured the variation within individual males was as great as that between males within a population. Since drumming bouts appear to be highly variable within individual males, it is unlikely that information about mate quality is being portrayed in these signals. However, due to the small sample sizes in this study, it is difficult to draw any conclusions about the role of female preference for male drumming signals. Future studies employing female choice experiments on varying male weta signals might reveal which components of the drumming may be involved in mate quality. In addition, it would be interesting to record *H. pallitarsis* drumming bouts from the Wellington region where it is sympatric with *H. bilobatus*, which also uses vibratory drumming in courtship displays. The theory of 'character displacement' predicts there will be increased selection where species with similar communication systems overlap in space (Brown & Wilson, 1956). However, sample sizes should be significantly large enough to ensure that the variation within males and within the populations is detected.

A second explanation to sexual selection that may account for the variation between populations found in this study is the effect of ecological factors, such as habitat characteristics and climate differences. One environmental aspect investigated in this study was ambient air temperature. The data revealed temperature significantly affected all drumming components measured, except for the mean number of oscillations per pulse. Drumming bout duration, number of pulses and inter-pulse interval all decreased with increasing temperature. Pulse rate increased with increasing temperature, a result that parallels that found in other Orthopterans (e.g. Jerusalem crickets, Weissman, 2001). It is well known that insects are cold blooded, and that their metabolism and activity is determined by their body temperature, which is in turn influenced by the surrounding air temperature (Mellanby, 1939). At low temperatures insect activity decreases. Palmerston North is approximately 400 km south of Coromandel and has a mean air temperature 2.1 °C lower than that recorded in Thames, a town 54 km south of Coromandel Town (New Zealand Meterological Service, 1983). Therefore, it is not surprising that drumming bouts from the colder Palmerston North populations were shorter, contained fewer pulses and subsequently had a lower pulse

rate than what was recorded in the northern Coromandel population. However, in order to accurately assess how temperature affects vibratory drumming in ground weta, future research should aim to record drumming bouts from geographically separated populations at the same temperature range and for a range of temperatures.

In conclusion, the pattern of vibratory drumming found among populations of *H. pallitarsis* matched that predicted by previous morphometric data and geographic proximity - smaller weta from the two geographically proximate Palmerston North populations had more similar drumming patterns when compared to the larger weta from Coromandel. Understanding the possible impact of different selection pressures on this mode of communication requires further research, not only into aspects of the male signals, but also investigating how females receive these signals and the interaction between both senders and receivers when communication underlies mate choice. Further study will help us to understand the factors contributing to the evolution of vibratory communication in New Zealand weta.

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Thesis Conclusions

Adult female *Hemiandrus pallitarsis* on the forest floor on Cuvier Island.

Thesis Conclusions

For the last fifty years there has been substantial debate concerning the definition of a species, and there have been at least 24 different species concepts named (Mayden, 1997). Much of argument seems to surround the difference between the concept of a species and actually inferring boundaries and numbers of species, that is, species delimitation (De Queiroz, 2007). Although there are attempts to form a single unifying species concept, several authors have stressed the importance of examining multiple different characters in order to gain evidence for the existence of separate species (De Queiroz, 2007; Roe & Sperling, 2007).

Accordingly, the aim of this thesis was to document the degree of morphological, genetic and behavioural variation within an endemic species of ground weta, *Hemiandrus pallitarsis*, in North Island, New Zealand. The results from all three characters provide no evidence of a species complex within *H. pallitarsis*, but suggest that it is morphologically, genetically and behaviourally variable across the North Island. In documenting the degree of variation within this species some interesting patterns were found. Specifically, it was identified that female weta are significantly larger than males, and weta in Palmerston North tend to be smaller than weta in northern Coromandel populations, possibly due to differences in temperature and season length between the two locations. Phylogenetic and phylogeographic analysis of *H. pallitarsis* populations across the North Island revealed substantial genetic structuring, large genetic distances, and a possible southern origin for this species. In fact, the results suggest *H. pallitarsis* populations have undergone range expansion in a south to north pattern, possibly following the pattern of land connection and uplift in the lower North Island during the late Pliocene – mid Pleistocene. Additionally, the application of the commonly used molecular clock calibration rate (1.4 – 2.6% sequence divergence per million years) to estimate possible divergence times of lineages within *H. pallitarsis*, was found to be problematic. This study highlighted the discrepancy in using a rate based on phylogenetic studies for a population-level study and found that divergence times tend to be overestimated when the invertebrate molecular clock is applied to *H. pallitarsis*

populations. The pre-copulatory drumming behaviour of male *H. pallitarsis* wetas was recorded in two adjacent populations and one distant population. Variation in the patterns of drumming supported the morphology/geographic proximity hypothesis – smaller weta in the two geographically proximate Palmerston North populations had more similar drumming behaviour patterns than larger weta from Coromandel – and did not match the patterns of genetic isolation previously found.

The different topics examined in this thesis illustrate the importance of using multiple characters coupled with geographic information when investigating the species status of an organism. Moreover, this thesis was able to provide some baseline data on the level of morphometric, genetic and behavioural variation that can be found within a single terrestrial invertebrate species in North Island, New Zealand.

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Appendix A. Correlation matrix for nine morphometric variables produced using 91 weta measurements. Values in bold show strongly correlated characters (> 90%).

	Pronotum length	Protibia length	Mesotibia length	Metatibia length	Metafemur length	Head width	Head length	Fastigium width	Body length
Pronotum length	1								
Protibia length	0.8213	1							
Mesotibia length	0.8099	**0.9574**	1						
Metatibia length	0.8201	**0.9264**	**0.9316**	1					
Metafemur length	0.8888	**0.9229**	**0.9220**	**0.9297**	1				
Head width	0.8954	0.7344	0.7248	0.7392	0.8245	1			
Head length	0.8996	0.7101	0.7045	0.7209	0.7987	**0.9386**	1		
Fastigium width	0.7425	0.5296	0.5117	0.4941	0.6187	0.8418	0.8119	1	
Body length	0.7962	0.5945	0.6092	0.6189	0.6998	0.8132	0.8268	0.7222	1

Appendix B. One sample *t*-test results produced using SPSS version 4.0 (SPSS, Chicago, Ill., USA) for eleven morphometric traits. Significant values at the $p < 0.05$ level are marked *

	Test Value = 0					
					95% Confidence Interval of the Difference	
Characters	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Pr	1.541	14	.146	.02600	-.0102	.0622
Mf	1.636	14	.124	.04067	-.0126	.0940
Fa	-.800	14	.437	-.01600	-.0589	.0269
BL	.341	14	.738	.03267	-.1729	.2382
Hw	.602	14	.557	.01800	-.0462	.0822
Hl	.359	14	.725	.01200	-.0597	.0837
Sg	-.516	14	.614	-.01333	-.0688	.0421
MxSg	1.024	14	.323	.03333	-.0365	.1032
MnSg	-3.400	7	.011*	-.05250	-.0890	-.0160
LAO	-2.169	5	.082	-.04000	-.0874	.0074
WAO	-2.282	5	.071	-.06500	-.1382	.0082

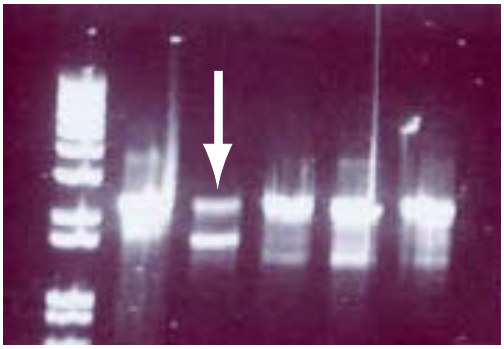
Appendix C. Principal components analysis results for male *H. pallitarsis.*

	PCA axis			
	1	2	3	4
Eigenvalue	5.4636	0.8931	0.5682	0.3959
Percent of total	0.683	0.112	0.071	0.049
Cumulative percent	0.683	0.795	0.866	0.915
Eigenvectors (component loadings)				
Pronotum	0.393	0.001	-0.261	-0.130
Metafemur	0.365	0.170	-0.386	-0.067
Fastigium	0.267	-0.612	0.643	-0.161
Body length	0.379	-0.072	-0.015	0.338
Head width	0.384	-0.248	-0.252	-0.136
Head length	0.405	-0.150	-0.129	-0.098
Subgenital plate	0.275	0.628	0.421	-0.556
Width subgenital plate	0.333	0.336	0.334	0.708

Appendix D. Principal components analysis results for female *H. pallitarsis.*

	PCA axis			
	1	2	3	4
Eigenvalue	5.1772	1.0752	0.6577	0.3901
Percent of total	0.647	0.134	0.082	0.049
Cumulative percent	0.647	0.782	0.864	0.913
Eigenvectors (component loadings)				
Pronotum	0.403	-0.252	-0.033	-0.180
Metafemur	0.402	-0.046	0.144	-0.289
Fastigium	0.339	-0.006	-0.563	0.580
Body length	0.291	-0.292	0.749	0.375
Head width	0.407	-0.141	-0.224	-0.087
Head length	0.410	-0.177	-0.113	-0.167
Subgenital plate	0.256	0.658	0.178	0.423
Width subgenital plate	0.278	0.604	0.073	-0.439

Appendix E. Agarose gel showing amplified DNA products. Arrow indicates product with two bands, where one is potentially a *Numt* sequence.



Appendix F. Number of individuals sequenced, number of haplotypes found, and number of each haplotype found at each location.

Location	No. of individuals	No. of haplotypes	Clade I																														
			Haplotype ID																														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Great Barrier Island	1	1	1																												
Little Barrier Island	2	2		1	1																										
Moehau	12	5				2	2	6	1	1																					
Cuvier Island	9	5									6	1	1	1																	
Taiharuru	5	1													5																
Middle Island	9	4														6	1	1	1												
Coromandel Town	8	5																		2	1	1	2	2							
Ruamahuanui Island	8	1																							1						
Kauaeranga Valley	8	7																								1	1	1	1	1	1
Cambridge	1	1																													
Taupo	2	1																													
Lake Waikaremoana	3	3																													
Taranaki	4	2																													
Wanganui	1	1																													
Palmerston North	9	8																													
Pohangina Valley	1	1																													
Wellington	9	8																													
Total	**92**	**56**																													

Appendix F. cont…

	Clade I cont.										Clade II								Clade III							
	Haplotype ID																									
	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
Great Barrier Island																										
Little Barrier Island																										
Moehau																										
Cuvier Island																										
Taiharuru																										
Middle Island																										
Coromandel Town																										
Ruamahuanui Island																										
Kauaeranga Valley	2																									
Cambridge											1															
Taupo												2														
Lake Waikaremoana		1	1	1																						
Taranaki													1	3												
Wanganui					1																					
Palmerston North						1	1	1	1						1	2	1	1								
Pohangina Valley										1																
Wellington																			2	1	1	1	1	1	1	1

Appendix G. Pairwise genetic distances based on COI sequence data. TVM + I + G above diagonal, observed distance below diagonal. Individual code names, sampling locations and haplotype codes are shown. Bold coloured boxes indicate clades in ML analysis (Fig. 3.10).

Code	Location	Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
GB	Great Barrier Island	1	I	0.05	0.06	0.04	0.04	0.04	0.04	0.05	0.07	0.08	0.08	0.07	0.04	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.05
GW226	Little Barrier Island	2	0.04		0.00	0.06	0.06	0.07	0.07	0.08	0.08	0.08	0.08	0.07	0.06	0.07	0.08	0.07	0.07	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.06
GW227	Little Barrier Island	3	0.04	0.00		0.07	0.06	0.07	0.07	0.08	0.08	0.08	0.08	0.07	0.06	0.08	0.08	0.07	0.08	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.06
CO144	Moehau	4	0.03	0.05	0.05		0.00	0.01	0.01	0.01	0.06	0.07	0.06	0.06	0.02	0.04	0.05	0.04	0.04	0.02	0.01	0.01	0.02	0.01	0.04	0.04	0.03	0.03	0.04
CO145	Moehau	5	0.03	0.04	0.05	0.00		0.01	0.01	0.01	0.06	0.06	0.06	0.06	0.02	0.04	0.05	0.04	0.05	0.02	0.01	0.01	0.02	0.02	0.04	0.04	0.03	0.03	0.04
CO148	Moehau	6	0.03	0.05	0.05	0.01	0.01		0.00	0.00	0.06	0.07	0.06	0.06	0.02	0.05	0.05	0.05	0.05	0.02	0.02	0.02	0.02	0.02	0.04	0.04	0.04	0.04	0.05
CO71	Moehau	7	0.04	0.05	0.05	0.01	0.01	0.00		0.00	0.06	0.07	0.06	0.06	0.02	0.05	0.05	0.04	0.05	0.02	0.01	0.02	0.02	0.02	0.04	0.04	0.04	0.04	0.04
CO73	Moehau	8	0.04	0.05	0.05	0.01	0.01	0.00	0.00		0.06	0.07	0.06	0.06	0.03	0.05	0.05	0.05	0.05	0.02	0.02	0.02	0.02	0.02	0.04	0.04	0.04	0.04	0.05
CU76	Cuvier Island	9	0.05	0.06	0.06	0.04	0.04	0.04	0.04	0.04		0.01	0.00	0.00	0.07	0.07	0.07	0.06	0.07	0.06	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.05	0.06
CU77	Cuvier Island	10	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.01		0.01	0.00	0.07	0.08	0.08	0.07	0.08	0.07	0.06	0.06	0.06	0.06	0.07	0.06	0.06	0.06	0.07
CU82	Cuvier Island	11	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.00	0.01		0.00	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.06	0.07	0.06	0.06	0.06	0.06
CU86	Cuvier Island	12	0.05	0.05	0.05	0.04	0.04	0.05	0.04	0.05	0.00	0.00	0.00		0.06	0.07	0.07	0.07	0.07	0.06	0.05	0.05	0.06	0.05	0.06	0.06	0.06	0.06	0.06
CO67	Taiharuru	13	0.03	0.05	0.05	0.02	0.02	0.02	0.02	0.02	0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.02	0.01	0.01	0.02	0.02	0.04	0.04	0.04	0.04	0.04
MI100	Middle Island	14	0.04	0.05	0.06	0.03	0.03	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.04		0.00	0.00	0.00	0.05	0.04	0.04	0.04	0.04	0.03	0.03	0.02	0.03	0.03
MI101	Middle Island	15	0.04	0.06	0.06	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.04	0.00		0.00	0.00	0.05	0.04	0.05	0.05	0.05	0.04	0.03	0.03	0.03	0.03
MI98	Middle Island	16	0.04	0.05	0.05	0.03	0.03	0.04	0.03	0.04	0.05	0.05	0.05	0.05	0.04	0.00	0.00		0.00	0.04	0.03	0.04	0.04	0.04	0.03	0.03	0.02	0.02	0.03
MI99	Middle Island	17	0.04	0.05	0.06	0.03	0.04	0.04	0.04	0.04	0.05	0.06	0.05	0.05	0.04	0.00	0.00	0.00		0.05	0.04	0.04	0.05	0.04	0.03	0.03	0.02	0.03	0.03
CO121	Coromandel Town	18	0.04	0.05	0.05	0.02	0.02	0.02	0.02	0.02	0.04	0.05	0.05	0.05	0.02	0.04	0.04	0.03	0.04		0.01	0.01	0.00	0.01	0.03	0.04	0.04	0.03	0.04
CO127	Coromandel Town	19	0.03	0.04	0.04	0.01	0.01	0.01	0.01	0.01	0.04	0.05	0.04	0.04	0.01	0.03	0.03	0.03	0.03	0.01		0.01	0.00	0.00	0.03	0.03	0.03	0.02	0.03
CO129	Coromandel Town	20	0.03	0.04	0.04	0.01	0.01	0.02	0.02	0.02	0.04	0.04	0.04	0.04	0.01	0.03	0.04	0.03	0.03	0.01	0.01		0.01	0.01	0.03	0.03	0.03	0.03	0.03
CO141	Coromandel Town	21	0.03	0.04	0.04	0.01	0.02	0.02	0.02	0.02	0.04	0.05	0.05	0.04	0.02	0.03	0.04	0.03	0.04	0.00	0.00	0.01		0.00	0.03	0.03	0.03	0.03	0.04
CO37	Coromandel Town	22	0.04	0.05	0.05	0.01	0.01	0.02	0.02	0.02	0.04	0.05	0.04	0.04	0.02	0.03	0.04	0.03	0.03	0.01	0.00	0.01	0.00		0.03	0.03	0.03	0.03	0.03
AL112	Ruamahuanui Island	23	0.03	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.05	0.05	0.05	0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03		0.02	0.02	0.01	0.02
KA119	Kauaeranga Valley	24	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.05	0.05	0.04	0.03	0.02	0.03	0.02	0.03	0.03	0.02	0.03	0.03	0.03	0.02		0.00	0.01	0.01
KA120	Kauaeranga Valley	25	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.05	0.05	0.04	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.03	0.03	0.03	0.01	0.00		0.01	0.00
KA87	Kauaeranga Valley	26	0.03	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.05	0.05	0.04	0.03	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.01	0.01	0.01		0.01
KA88	Kauaeranga Valley	27	0.04	0.04	0.04	0.03	0.03	0.04	0.03	0.04	0.04	0.05	0.05	0.05	0.03	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.01	0.00	0.01	
KA89	Kauaeranga Valley	28	0.03	0.05	0.05	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.03	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.02	0.01	0.01	0.01	0.01
KA90	Kauaeranga Valley	29	0.03	0.05	0.05	0.03	0.03	0.03	0.03	0.03	0.05	0.05	0.05	0.05	0.03	0.03	0.03	0.02	0.03	0.03	0.02	0.03	0.03	0.03	0.01	0.01	0.01	0.00	0.01
KA91	Kauaeranga Valley	30	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.05	0.05	0.04	0.03	0.02	0.03	0.02	0.02	0.03	0.02	0.03	0.03	0.03	0.01	0.00	0.00	0.01	0.00
GW230	Lake Waikaremoana	31	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.06	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05
GW231	Lake Waikaremoana	32	0.05	0.05	0.06	0.05	0.05	0.05	0.06	0.06	0.05	0.05	0.05	0.05	0.06	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
LW189	Lake Waikaremoana	33	0.05	0.05	0.06	0.05	0.05	0.05	0.06	0.06	0.05	0.05	0.05	0.05	0.06	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
GW190	Wanganui	34	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.06	0.03	0.04	0.03	0.03	0.06	0.06	0.06	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
GW92A	Palmerston North	35	0.04	0.05	0.05	0.04	0.04	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
PN178	Palmerston North	36	0.04	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.04	0.05	0.05	0.04	0.05	0.05	0.05	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
PN22	Palmerston North	37	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
PN23	Palmerston North	38	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
GW87	Pohangina Valley	39	0.04	0.05	0.06	0.04	0.04	0.05	0.05	0.05	0.04	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05
GW171	Cambridge	40	0.06	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.07	0.07	0.06	0.07	0.06	0.06	0.08	0.07	0.07	0.08	0.08	0.08	0.07	0.07	0.07	0.07
GW200	Taupo	41	0.06	0.08	0.09	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.08
GW235	Taranaki	42	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.06	0.05	0.05	0.05	0.05	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.06	0.06	0.06
TK187	Taranaki	43	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.07	0.07	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.07	0.07
GW232	Palmerston North	44	0.07	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.08	0.08	0.07	0.07	0.07	0.07	0.07
PN174	Palmerston North	45	0.07	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.08	0.08	0.07	0.07	0.07	0.07	0.07
PN176	Palmerston North	46	0.07	0.07	0.08	0.07	0.07	0.08	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.07	0.08	0.08	0.07	0.07	0.07	0.07	0.07
PN177	Palmerston North	47	0.06	0.07	0.07	0.07	0.06	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.07	0.07	0.07
GW80	Wellington	48	0.08	0.08	0.08	0.07	0.07	0.07	0.08	0.08	0.07	0.06	0.07	0.07	0.08	0.08	0.08	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.08
GW82	Wellington	49	0.07	0.08	0.08	0.07	0.07	0.08	0.08	0.08	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.08
GW89A	Wellington	50	0.08	0.08	0.07	0.07	0.08	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.08	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.08
GW89B	Wellington	51	0.08	0.08	0.07	0.07	0.07	0.06	0.06	0.07	0.06	0.06	0.06	0.06	0.08	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.08
WN181	Wellington	52	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.07	0.06	0.08	0.07	0.07	0.07	0.07	0.08	0.08	0.07	0.08	0.08	0.08	0.08	0.08	0.07	0.08
WN182	Wellington	53	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.07	0.06	0.08	0.07	0.07	0.07	0.08	0.08	0.08	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08
WN183	Wellington	54	0.08	0.08	0.07	0.08	0.07	0.07	0.07	0.07	0.06	0.06	0.07	0.06	0.08	0.08	0.08	0.07	0.08	0.08	0.08	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08
GW176	Wellington	55	0.07	0.08	0.08	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.08

Appendix G. cont…

Code	Location	Haplotype	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
GB	Great Barrier Island	1	0.04	0.04	0.04	0.07	0.07	0.07	0.07	0.05	0.05	0.06	0.06	0.06	0.10	0.10	0.08	0.10	0.11	0.11	0.11	0.10	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
GW226	Little Barrier Island	2	0.06	0.06	0.05	0.07	0.08	0.07	0.07	0.06	0.07	0.07	0.07	0.07	0.14	0.16	0.09	0.11	0.14	0.12	0.13	0.11	0.13	0.13	0.12	0.12	0.12	0.12	0.13	0.13
GW227	Little Barrier Island	3	0.06	0.06	0.05	0.07	0.08	0.08	0.07	0.06	0.06	0.06	0.07	0.08	0.15	0.16	0.10	0.11	0.15	0.13	0.14	0.12	0.14	0.14	0.11	0.11	0.11	0.12	0.12	0.13
CO144	Moehau	4	0.04	0.04	0.04	0.07	0.08	0.08	0.08	0.06	0.06	0.06	0.06	0.06	0.11	0.12	0.09	0.11	0.11	0.11	0.11	0.10	0.11	0.11	0.12	0.11	0.11	0.12	0.12	0.11
CO145	Moehau	5	0.04	0.04	0.04	0.07	0.07	0.07	0.08	0.05	0.06	0.06	0.06	0.06	0.11	0.12	0.09	0.10	0.11	0.11	0.11	0.10	0.11	0.11	0.12	0.11	0.11	0.11	0.12	0.10
CO148	Moehau	6	0.04	0.04	0.04	0.07	0.08	0.08	0.07	0.06	0.06	0.06	0.07	0.06	0.11	0.13	0.09	0.11	0.12	0.12	0.13	0.11	0.11	0.12	0.10	0.09	0.10	0.10	0.11	0.11
CO71	Moehau	7	0.04	0.04	0.04	0.07	0.08	0.08	0.08	0.06	0.06	0.06	0.06	0.07	0.12	0.13	0.09	0.11	0.12	0.12	0.12	0.11	0.12	0.12	0.10	0.10	0.10	0.10	0.11	0.11
CO73	Moehau	8	0.04	0.04	0.04	0.07	0.09	0.09	0.08	0.06	0.06	0.06	0.07	0.07	0.12	0.13	0.10	0.12	0.12	0.12	0.13	0.11	0.12	0.12	0.11	0.10	0.10	0.11	0.11	0.12
CU76	Cuvier Island	9	0.05	0.06	0.05	0.07	0.07	0.07	0.04	0.05	0.05	0.06	0.06	0.06	0.13	0.14	0.11	0.13	0.11	0.11	0.12	0.11	0.10	0.11	0.09	0.09	0.10	0.10	0.10	0.11
CU77	Cuvier Island	10	0.06	0.07	0.06	0.07	0.08	0.08	0.04	0.06	0.06	0.06	0.06	0.06	0.14	0.15	0.11	0.13	0.12	0.12	0.13	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CU82	Cuvier Island	11	0.05	0.06	0.06	0.07	0.08	0.08	0.04	0.06	0.06	0.06	0.06	0.06	0.14	0.15	0.11	0.13	0.12	0.12	0.13	0.11	0.11	0.11	0.10	0.10	0.10	0.11	0.11	0.11
CU86	Cuvier Island	12	0.05	0.06	0.06	0.06	0.07	0.07	0.04	0.05	0.05	0.05	0.06	0.06	0.13	0.14	0.10	0.12	0.11	0.11	0.11	0.10	0.10	0.10	0.10	0.10	0.09	0.10	0.10	0.10
CO67	Taiharuru	13	0.04	0.04	0.04	0.07	0.08	0.08	0.08	0.07	0.06	0.06	0.06	0.07	0.11	0.13	0.09	0.11	0.12	0.12	0.13	0.11	0.12	0.12	0.13	0.12	0.12	0.13	0.13	0.12
MI100	Middle Island	14	0.02	0.03	0.03	0.08	0.07	0.07	0.08	0.06	0.06	0.06	0.06	0.08	0.10	0.11	0.07	0.09	0.11	0.11	0.11	0.10	0.12	0.12	0.11	0.11	0.11	0.12	0.12	0.12
MI101	Middle Island	15	0.02	0.03	0.03	0.08	0.08	0.08	0.08	0.06	0.06	0.06	0.06	0.08	0.11	0.12	0.08	0.10	0.11	0.11	0.11	0.10	0.12	0.12	0.11	0.11	0.11	0.12	0.12	0.12
MI98	Middle Island	16	0.02	0.03	0.02	0.07	0.07	0.07	0.08	0.05	0.06	0.06	0.06	0.08	0.10	0.11	0.08	0.09	0.11	0.11	0.11	0.10	0.12	0.12	0.10	0.10	0.11	0.11	0.12	0.11
MI99	Middle Island	17	0.03	0.03	0.03	0.08	0.08	0.08	0.08	0.06	0.06	0.06	0.06	0.08	0.11	0.12	0.08	0.09	0.11	0.11	0.12	0.11	0.12	0.13	0.11	0.11	0.12	0.12	0.13	0.12
CO121	Coromandel Town	18	0.04	0.04	0.04	0.06	0.07	0.07	0.07	0.06	0.05	0.05	0.05	0.07	0.14	0.15	0.10	0.12	0.13	0.13	0.14	0.12	0.13	0.14	0.13	0.13	0.14	0.14	0.14	0.14
CO127	Coromandel Town	19	0.03	0.03	0.03	0.06	0.07	0.07	0.07	0.06	0.05	0.05	0.05	0.06	0.12	0.14	0.10	0.11	0.12	0.12	0.13	0.12	0.13	0.13	0.13	0.12	0.12	0.13	0.13	0.12
CO129	Coromandel Town	20	0.03	0.03	0.03	0.07	0.08	0.08	0.07	0.06	0.05	0.05	0.05	0.06	0.12	0.13	0.09	0.11	0.12	0.12	0.12	0.11	0.12	0.12	0.12	0.11	0.11	0.11	0.12	0.12
CO141	Coromandel Town	21	0.04	0.03	0.03	0.06	0.06	0.07	0.06	0.05	0.05	0.05	0.05	0.06	0.14	0.14	0.10	0.12	0.13	0.13	0.13	0.12	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.13
CO37	Coromandel Town	22	0.03	0.03	0.03	0.06	0.07	0.07	0.07	0.05	0.05	0.05	0.05	0.06	0.13	0.15	0.10	0.12	0.13	0.13	0.14	0.12	0.12	0.12	0.13	0.12	0.13	0.13	0.12	0.12
AL112	Ruamahuanui Island	23	0.02	0.02	0.02	0.07	0.07	0.08	0.08	0.05	0.05	0.05	0.05	0.07	0.13	0.14	0.11	0.12	0.12	0.11	0.11	0.10	0.13	0.13	0.12	0.12	0.13	0.13	0.14	0.12
KA119	Kauaeranga Valley	24	0.01	0.02	0.00	0.07	0.07	0.07	0.07	0.05	0.05	0.05	0.05	0.07	0.12	0.13	0.08	0.10	0.12	0.12	0.12	0.11	0.12	0.12	0.11	0.11	0.12	0.12	0.13	0.12
KA120	Kauaeranga Valley	25	0.01	0.01	0.00	0.07	0.06	0.06	0.07	0.05	0.05	0.05	0.05	0.07	0.11	0.13	0.09	0.10	0.11	0.11	0.12	0.11	0.12	0.12	0.11	0.11	0.12	0.13	0.13	0.11
KA87	Kauaeranga Valley	26	0.01	0.00	0.01	0.06	0.07	0.07	0.07	0.05	0.05	0.05	0.05	0.07	0.11	0.13	0.09	0.11	0.11	0.11	0.12	0.11	0.11	0.12	0.11	0.11	0.12	0.12	0.13	0.11
KA88	Kauaeranga Valley	27	0.01	0.01	0.00	0.07	0.07	0.07	0.07	0.05	0.05	0.05	0.05	0.08	0.12	0.13	0.10	0.12	0.12	0.12	0.12	0.11	0.13	0.13	0.12	0.12	0.13	0.13	0.14	0.12
KA89	Kauaeranga Valley	28		0.01	0.01	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.06	0.11	0.12	0.09	0.11	0.10	0.10	0.11	0.10	0.11	0.11	0.11	0.10	0.11	0.12	0.12	0.11
KA90	Kauaeranga Valley	29	0.01		0.01	0.07	0.08	0.08	0.08	0.06	0.05	0.06	0.05	0.07	0.12	0.13	0.10	0.12	0.12	0.12	0.12	0.11	0.12	0.12	0.12	0.11	0.12	0.13	0.13	0.12
KA91	Kauaeranga Valley	30	0.01	0.01		0.07	0.07	0.07	0.07	0.05	0.05	0.05	0.05	0.07	0.11	0.13	0.09	0.11	0.11	0.11	0.12	0.11	0.12	0.12	0.11	0.11	0.12	0.13	0.13	0.12
GW230	Lake Waikaremoana	31	0.05	0.05	0.05		0.02	0.02	0.07	0.05	0.06	0.06	0.05	0.07	0.11	0.12	0.10	0.12	0.14	0.14	0.15	0.13	0.11	0.11	0.12	0.12	0.11	0.12	0.12	0.11
GW231	Lake Waikaremoana	32	0.05	0.05	0.05	0.01		0.00	0.07	0.05	0.06	0.06	0.06	0.06	0.12	0.12	0.11	0.12	0.12	0.13	0.13	0.12	0.12	0.12	0.11	0.12	0.12	0.13	0.12	0.12
LW189	Lake Waikaremoana	33	0.05	0.05	0.05	0.01	0.00		0.07	0.05	0.07	0.07	0.07	0.06	0.11	0.12	0.10	0.11	0.13	0.13	0.14	0.12	0.12	0.12	0.11	0.11	0.11	0.12	0.12	0.11
GW190	Wanganui	34	0.05	0.06	0.05	0.05	0.05	0.05		0.06	0.06	0.06	0.06	0.06	0.15	0.17	0.10	0.12	0.14	0.13	0.13	0.13	0.13	0.13	0.10	0.11	0.11	0.12	0.11	0.14
GW92A	Palmerston North	35	0.04	0.04	0.04	0.04	0.04	0.04	0.05		0.02	0.02	0.02	0.05	0.11	0.11	0.10	0.11	0.11	0.11	0.11	0.10	0.09	0.09	0.11	0.10	0.11	0.10	0.10	0.09
PN178	Palmerston North	36	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.01		0.00	0.01	0.05	0.12	0.12	0.11	0.12	0.13	0.12	0.12	0.11	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10
PN22	Palmerston North	37	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.02	0.00		0.01	0.06	0.12	0.12	0.11	0.12	0.13	0.12	0.13	0.12	0.10	0.10	0.11	0.10	0.10	0.11	0.11	0.10
PN23	Palmerston North	38	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.02	0.01	0.01		0.06	0.11	0.12	0.10	0.12	0.12	0.11	0.12	0.11	0.08	0.09	0.11	0.10	0.10	0.10	0.10	0.09
GW87	Pohangina Valley	39	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04		0.13	0.14	0.11	0.13	0.12	0.11	0.12	0.11	0.11	0.11	0.10	0.10	0.10	0.10	0.10	0.11
GW171	Cambridge	40	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.07	0.07	0.07	II	0.00	0.04	0.05	0.06	0.06	0.07	0.05	0.09	0.10	0.09	0.09	0.10	0.10	0.10	0.09
GW200	Taupo	41	0.07	0.08	0.07	0.07	0.07	0.07	0.09	0.07	0.07	0.07	0.07	0.08	0.00		0.05	0.06	0.07	0.07	0.07	0.06	0.11	0.11	0.11	0.11	0.11	0.11	0.12	0.10
GW235	Taranaki	42	0.06	0.07	0.06	0.07	0.07	0.07	0.07	0.06	0.07	0.07	0.07	0.07	0.03	0.04		0.01	0.08	0.07	0.08	0.06	0.09	0.09	0.07	0.08	0.07	0.08	0.08	0.08
TK187	Taranaki	43	0.07	0.07	0.07	0.07	0.08	0.07	0.08	0.07	0.08	0.08	0.08	0.08	0.04	0.04	0.01		0.09	0.08	0.08	0.07	0.11	0.11	0.08	0.09	0.09	0.09	0.09	0.10
GW232	Palmerston North	44	0.06	0.07	0.07	0.08	0.07	0.08	0.08	0.07	0.08	0.08	0.07	0.07	0.05	0.05	0.06	0.06		0.01	0.01	0.01	0.13	0.13	0.13	0.12	0.13	0.13	0.14	0.11
PN174	Palmerston North	45	0.06	0.07	0.07	0.08	0.07	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.05	0.05	0.05	0.06	0.01		0.00	0.01	0.12	0.12	0.13	0.13	0.13	0.13	0.14	0.11
PN176	Palmerston North	46	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.07	0.07	0.08	0.07	0.07	0.05	0.05	0.05	0.06	0.01	0.00		0.01	0.12	0.13	0.13	0.13	0.12	0.13	0.13	0.11
PN177	Palmerston North	47	0.06	0.07	0.07	0.08	0.07	0.07	0.08	0.06	0.07	0.07	0.07	0.07	0.04	0.04	0.05	0.05	0.01	0.01	0.01		0.11	0.12	0.11	0.11	0.11	0.12	0.12	0.10
GW80	Wellington	48	0.07	0.08	0.08	0.07	0.08	0.08	0.08	0.06	0.06	0.07	0.06	0.07	0.06	0.07	0.06	0.07	0.08	0.08	0.08	0.07	III	0.00	0.07	0.06	0.07	0.07	0.07	0.00
GW82	Wellington	49	0.07	0.08	0.08	0.07	0.08	0.08	0.08	0.06	0.07	0.07	0.06	0.07	0.06	0.07	0.06	0.07	0.08	0.08	0.08	0.07	0.00		0.07	0.07	0.07	0.07	0.07	0.01
GW89A	Wellington	50	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.05	0.06	0.08	0.08	0.08	0.07	0.05	0.05		0.00	0.01	0.01	0.01	0.06
GW89B	Wellington	51	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.07	0.06	0.06	0.08	0.08	0.08	0.07	0.05	0.05	0.00		0.01	0.00	0.01	0.06
WN181	Wellington	52	0.07	0.08	0.08	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.07	0.05	0.06	0.08	0.08	0.08	0.07	0.05	0.05	0.01	0.01		0.00	0.00	0.06
WN182	Wellington	53	0.07	0.08	0.08	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.06	0.06	0.08	0.08	0.08	0.07	0.05	0.05	0.01	0.00	0.00		0.00	0.06
WN183	Wellington	54	0.08	0.08	0.08	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.08	0.08	0.08	0.08	0.05	0.05	0.01	0.01	0.00	0.00		0.07
GW176	Wellington	55	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.06	0.07	0.07	0.06	0.07	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.07	0.00	0.01	0.05	0.04	0.05	0.05	0.05	