Evolution of diversity: analysis of species and speciation in *Hemiandrus* ground wētā

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In loving memory of my grandad,
Bruce Smith

> “The important thing is not to stop questioning. Curiosity has its own reason for existing. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvellous structure of reality. It is enough if one tries merely to comprehend a little of this mystery every day. Never lose a holy curiosity.”
>
> - Albert Einstein

Abstract

Patterns of biodiversity and endemism in New Zealand are explored, with a focus on the ground wētā genus *Hemiandrus*. I first investigated factors that determined regional levels of endemism using a generalised linear model based on analysis of 2322 species of endemic New Zealand invertebrates. I found that widespread species are uncommon in New Zealand and most invertebrates occupied few regions. Number of endemic species per region was positively correlated with total number of species and size of the region 3 million years ago. Within one clade of *Hemiandrus* I found that North and South Islands differed in how they were occupied: South Island had many species with small non-overlapping ranges, whereas North Island was largely dominated by a single species. This is likely due to differences in age of different parts of New Zealand, yet this pattern was absent in another clade of ground wētā species, showing that properties of species themselves also have a large impact on species ranges and speciation.

I applied several strategies to the *Hemiandrus maculifrons* species complex to test putative species boundaries (chapter 3). I compared morphological methods (Gaps in Continuous Characters across Geography (GCCG)) and genetic methods (Bayesian Species Delimitation, Rosenberg's P(AB), P(Randomly Distinct), P ID(Liberal)). Some of these strategies indicated that all or nearly all mtDNA clades tested represented separate species, while others indicated that no clades were likely to be distinct species. I concluded that *H. maculifrons* comprises three species (plus an under-sampled microendemic species, chapter 4); a conclusion that is discordant with the results of the "species delimitation" methods but consistent with other genetic, morphological and distributional data.

Since the genus *Hemiandrus* was thought to comprise only nine named species but dozens of alleged species, I tested whether the purported diversity accurately reflected biological diversity in the genus or whether it was exaggerated due to speculative classification (chapter 5). To do this, I applied traditional techniques to search for qualitative or quantitative differences between individuals using a model where species are separately evolving lineages that form separate genotypic clusters with no or few intermediates when in contact (Mallet 1995). Most proposed operational taxonomic units were supported, but some names appear to be synonymies while others appear to encompass more diversity than previously recognised. I concluded that *Hemiandrus* comprises at least 25 species, but as specimens representing all tag-names[1]

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[1] A tag-name is an informal name that indicates an entity that may be a separate species, monophyletic group or separate interbreeding population of uncertain taxonomic rank (Leschen *et al*. 2009).

were not available, additional diversity may exist within *Hemiandrus* than recognised here.

Phylogenetic analysis of mtDNA sequences identified two major clades within New Zealand *Hemiandrus*. Using nuclear markers and morphological traits I found strong support for these two clades. Derived shared traits were identified that can determine to which clade each species belongs. Concordance between genetic markers (four loci) and morphology resolved evolutionary relationships from which I propose dividing the group into two separate genera.

Preface

The overall aim of this research project, "Evolution of diversity: analysis of species and speciation in *Hemiandrus* ground wētā", was to examine patterns of biodiversity and endemism in New Zealand, focusing particularly on the genus *Hemiandrus* (ground wētā). This genus has been suggested to comprise high species diversity and many taxa with restricted ranges, but diversity within this genus is largely unquantified.

In chapter one I explore the patterns and drivers of microendemism in New Zealand invertebrates. The finding that widespread species are uncommon in New Zealand and most invertebrates occupied few regions led me to question why a single ground wētā species, *H. maculifrons*, is found so extensively throughout both main islands of New Zealand (chapter 3). I explored this using multiple lines of evidence, including nuclear markers developed in chapter 2 with the help of Eddie Dowle, who sequenced multiple anostostomatid mtDNA genomes for her PhD thesis (2013). My results from chapter 3 suggest that *H. maculifrons* comprises multiple species and these are described in chapter 4 along with two morphologically similar species.

In chapter 5 I assess the overall purported species diversity within *Hemiandrus*. I first examined specimens collected during extensive field work and from assorted collections, in order to gain an understanding of morphological variation within the genus. Based on this background knowledge I was then able to match specimens to tag-names. Most tag-names do reflect biological diversity, although some appear to be synonymies, while others may comprise several species. Two of these tag-named species are described in chapter 6: Taylor Smith, B. L., Morgan-Richards, M., & Trewick, S. A. (2013). New Zealand ground wētā (Anostostomatidae: *Hemiandrus*): descriptions of two species with notes on their biology. *New Zealand Journal of Zoology*, *40*(4), 314-329).

In chapter 7 I show that the high species diversity within *Hemiandrus* compared to other New Zealand anostostomatid genera may be partially explained by ground wētā comprising multiple morphologically and genetically distinct genera.

These chapters are intended for publication and so references are presented at the end of each chapter.

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Chapter 1. Microendemism in New Zealand

Introduction

Variation in diversity and endemism

As recognised by Alfred Wallace (1876), diversity is not evenly distributed around the globe. An example of this is the observation that there is more variation in animal life at the tropics than in temperate regions (latitudinal biodiversity gradient: LBG) (Dowle *et al.* 2013). Besides an uneven distribution in diversity worldwide, there is also variation in endemism levels in that alpha diversity may be similar among regions but differs as a function of the degree of endemism. Like diversity, endemism shows a pattern of decreasing levels with increasing latitude (Jablonski, *et al.* 2006), although some temperate regions, e.g. New Zealand, have high endemism and can thus be considered 'biodiversity hotspots' (Myers *et al.* 2000).

One approach to understanding the uneven distribution of diversity focusses on colonisation and extinction rates. MacArthur and Wilson's (1967) model was founded on the idea that an area could contain a finite number of species, determined by an equilibrium between the arrival of new taxa and extinction, and by land area, with larger islands capable of containing more species. Other factors such as distance from sources and degree of isolation also affect the number of species. Likewise, these factors control endemism, but island species can only become endemic by the extinction of the species from the source, by the divergence (i.e. speciation) of the island population from the source population and/or by sympatric speciation on the island. Therefore it has been suggested that large islands may have higher endemism due to greater speciation rates, as a result of both size and heterogeneity effects (Kisel and Barraclough 2010). Higher endemism on large islands might also result from reduced extinction rates because species with greater ranges have lower extinction rates (Crampton *et al.* 2010; Foote *et al.* 2007).

Diversity and endemism does not only vary among islands, but also within islands and landmasses. Well-known examples include the Caucasus region on the border of Europe and Asia, and South Africa's Cape Floristic Province, two areas which have extremely high endemism compared to the land that they neighbour. Madagascar and New Caledonia are islands recognised as having high levels of endemism (Myers *et al.* 2000), but both of these islands also have high microendemism: regions within the islands that contain a high number of range restricted taxa (Nattier *et al.* 2012; Wilmé *et al.* 2006) (Fig. 1.1). The processes that result in endemism on islands also create microendemism in regions that are apparently not isolated: extinction outside the region and the divergence of populations within and outside of the region. However, when regions are not geographically isolated, range restriction and range changes are also likely to be important factors.

Rather than being fixed, range changes are part of the life cycle of a species, and a property that can be heritable (Jablonski 2000; Webb and Gaston 2003). Foote *et al.* (2007) found that extinct marine molluscs underwent range expansion and an increase in abundance to peak at either early or mid-duration of the species lifetime and then decline to extinction. Similarly, Nattier et al (2012) found that within the microendemic species of the New Caledonian cricket genus, *Agnotecous*, range size was correlated with species age. Therefore it is likely that microendemism might be a temporary state and the species may have yet to expand to inhabit adjacent regions. Although time frames are not well understood, it is likely that range change is more rapid than speciation so in the short term could be expected to have a stronger effect on regional diversity (Anderson 1994). Within regions of high endemism, distributional patterns appear to have remained stable for long periods, so it seems that range size is also strongly influenced by properties of the region. Jansson (2003) concluded that low oscillations in climate change in different geopolitical units of the world correlated with lower rates of extinction and so higher endemism of vascular plants and various animals. However, although there are global trends in biodiversity patterns, there are many instances where local circumstances are influential. For example range size in birds tends to be smallest on islands and mountainous areas (Orme *et al.* 2006). Anthropogenic changes such as deforestation and the introduction of predators/competitors, have had extraordinary short-term effects on biodiversity, as has been witnessed in New Zealand, so are likely to lead to changes in modern distributional patterns and thus also variation in endemism among regions.

Microendemism in New Zealand

In Europe, species diversity and endemism is concentrated in the south (Baquero and Tellería 2001; Essl *et al.* 2013), resulting in the Mediterranean region being one of the most biodiverse areas in the world (Myers *et al.* 2000) (Fig. 1.2). It is likely that the low latitude of this area made it less prone to glaciation and thus the extinction experienced by more northern parts of Europe during the Pleistocene. Although New Zealand is much smaller than Europe, there is

Figure 1.1. Madagascar is an island with high endemism compared to the global average and variable levels of microendemism. Coloured regions represent centres of microendemism, adapted from Wilmé *et al.* (2006).

reason to expect that patterns of microendemism may be more complicated than simply high endemism close to the equator and low endemism in regions closer to the poles. Firstly, New Zealand is an island with a high endemism level overall making it more similar to other islands with high endemism including Madagascar and New Caledonia that also have prominent patterns of microendemism. On these islands, microendemism has been attributed to the effects of habitat heterogeneity (Nattier *et al.* 2012) and past climate change (Wilmé *et al.* 2006). Secondly, New Zealand has a geologically active landscape and research has showed that its biota has responded to this resulting in complex phylogeographic patterns (Trewick and Bland 2011). Diversity is not simply graded latitudinally from high to low as in Europe (Hewitt 1996, 1999; Taberlet *et al.* 1998; Wallis and Trewick 2009). Therefore, it might be expected that endemism in New Zealand is distributed in a complex manner.

An examination of the distribution of plant endemism in New Zealand indicated that it was not evenly graded across the landscape. Wardle (1963) divided New Zealand into five arbitrary regions based on numbers of endemic vascular plants and showed that three regions have high endemism and two regions have low endemism (Fig. 1.3A). Wardle (1963) also described how some taxa have distributions that include some of these regions of high endemism but not the intervening areas. These two patterns of range disjunction and range restriction (i.e. microendemism) are likely to be caused by different processes acting on different timescales. A disjunct range is likely to be the result of shorter timescale processes, whereas a protracted disjunction could lead to microendemism because of continued evolutionary divergence or extinction from all but one of the occupied areas.

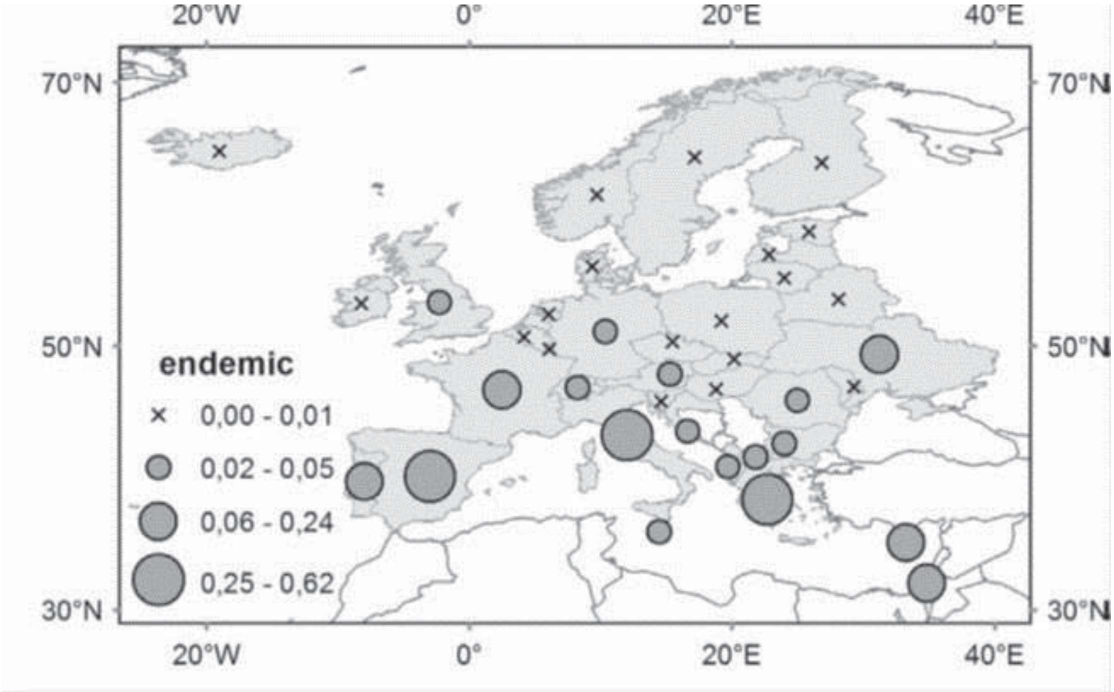

Figure 1.2. Endemism in Europe is higher at more southerly latitudes due to Pleistocene glaciation. Endemism was calculated by combining the species numbers of nine taxonomic groups (vascular plants, bryophytes, mammals, birds, fish, reptiles, amphibians, dragonflies, grasshoppers). The effect of different absolute species numbers between taxonomic groups were eliminated by calculating the relative proportion of species numbers in a country relative to the highest species number within this taxonomic group in any country of the dataset. Then cross-taxon endemism indices per country were calculated by averaging these proportions across all taxonomic groups. From Essl *et al.* (2013).

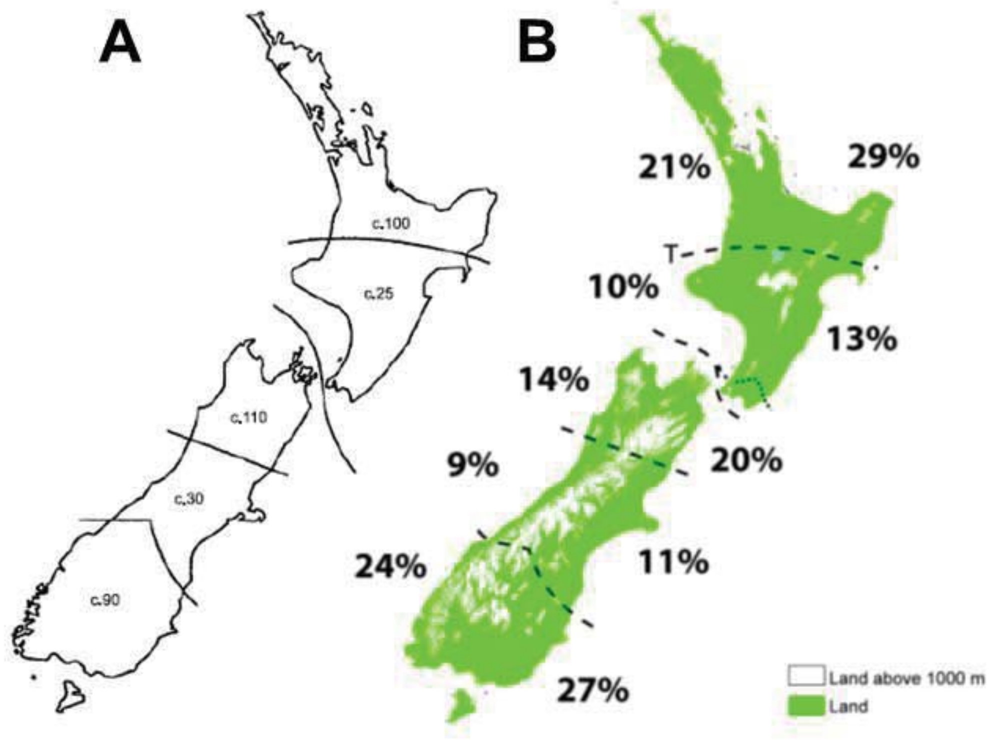

Figure 1.3. New Zealand has variation in levels of endemism between regions. **A.** Approximate numbers of endemic vascular plants in each of the five regions, based on Wardle (1963). **B.** Insect endemism in Wardle regions expressed as the percent of species in a region that are endemic to region (left; 1724 species total), and percentage of endemics that are endemic to a particular region (right; 596 species), from Trewick *et al*. (2011).

Other research using plant distribution maps has supported patterns like those illustrated by Wardle (1963) (Burrows 1965; Cockayne 1928; Connor 2002; Druce 1984; McGlone 1985; Wardle 1991). Wardle regions appear to correspond to diversity in insect data too (Fig. 1.3B) (Craw 1988; Trewick *et al.* 2011). Endemism has also been mapped using different boundaries such as 1 degree grid squares (Heads 1997), alpine regions (McGlone *et al.* 2001) and botanical regions (Rogers and Walker 2005). In many instances taxon ranges approximate to regions but extend someway beyond the boundaries of the predefined regions (e.g. Connor, 2002). This may indicate that the limits of regions of high endemism are arbitrary, but range limits are unlikely to coincide because species' range is a dynamic trait. Microendemism in New Zealand has been attributed to various geological, climatic and ecological processes. For example, Wardle (1963) proposed that areas of high endemism were refugia during Pleistocene glaciation. In contrast, others have suggested that in some regions endemism has been limited by availability of terrestrial habitat prior to the Pleistocene (Rogers 1989; Trewick and Bland 2011).

Fauna of New Zealand series and testing endemism in New Zealand

Previous observations and studies of variation in endemism among New Zealand regions have mostly focused on plants, although Craw (1988) and Heads (1997) presented selected examples of particular distribution patterns of insects. Trewick et al (2011) collated total data for a random subset of available invertebrate data using the Fauna of New Zealand series/Ko te Aitanga Pepeke o Aotearoa (FNZ). The FNZ is a monograph series (1982-) summarising data on some of New Zealand's invertebrate fauna. Each FNZ issue contains information on the distribution of the species described within and records their occurrence across New Zealand. Location data are linked to standardised geographic regions (Crosby regions) of similar area defined by at least some natural features of the landscape such as water catchments, rivers and climatic features (Crosby *et al.* 1998; Crosby *et al.* 1976) (Fig. 1.4A, Table 1.1). These regions were designed primarily as a basis for recording insect collecting sites, aiding the sorting and documentation of specimens (Crosby *et al.* 1976). The sampling within the FNZ series has greatly improved over time and now represents a valuable resource for the analysis of microendemism in New Zealand.

In order to test whether endemism varies among regions it is important that the data are independent of sampling effort. The discovery of new species within a region might encourage more search effort there and so drive the discovery of yet more putative microendemics that are not in fact restricted to the region in question. By comparing patterns of endemism among well-sampled taxa with patterns in the total dataset the influence of sampling effort effects should be distinguishable from true underlying microendemism.

If the data are not dominated by signal from variation in sampling effort, they can be used to determine whether there is evidence for regional variation in diversity and endemism in invertebrates. If microendemism is not a real feature of the environment, homogenous levels of endemism among regions would be the expectation. Furthermore, if microendemism is uncommon and being widespread is the common state then one would expect that most taxa would be found in all regions and very few in a single region (Fig. 1.5). If microendemism is common, it would be expected that most taxa would be found within one or few adjacent regions and few taxa found in most or all regions. It will also be possible to quantify the degree of overlap between endemism observed in this study and previous studies and using a set of environmental variables to test for correlates, it should be possible to identify potential drivers of microendemism.

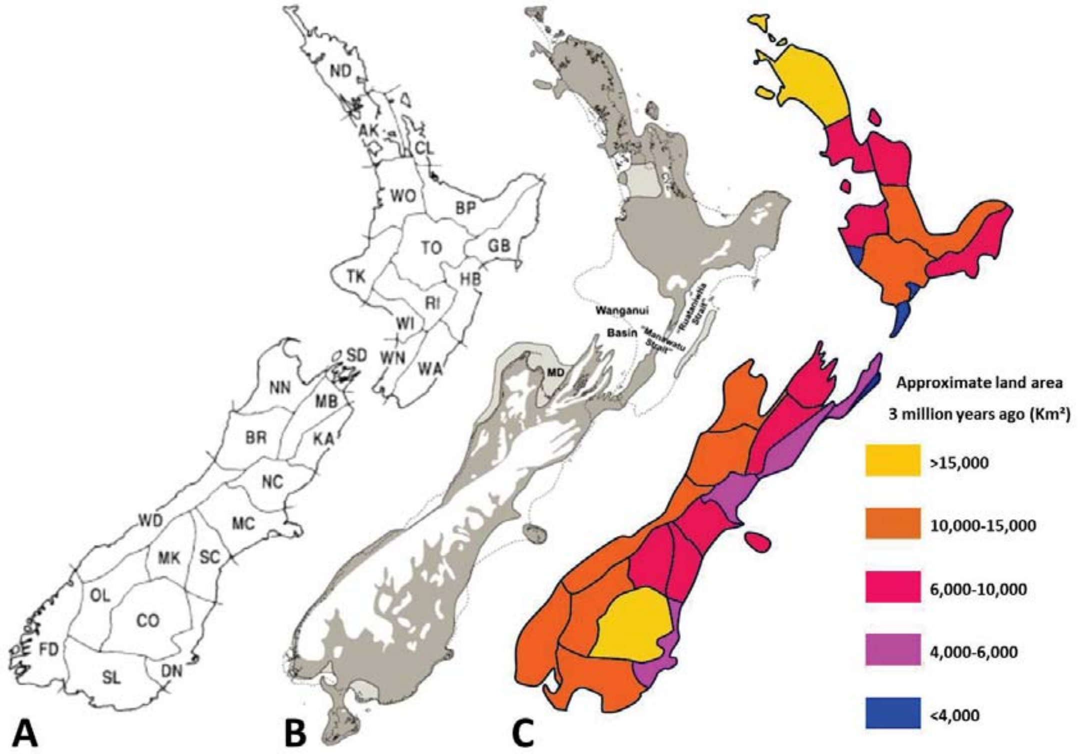

Figure 1.4. A. Twenty-eight entomological regions of New Zealand used to investigate microendemism and area codes and boundaries used to categorise specimen locality data (after Crosby *et al*. 1976). For full names of regions see Table 1.1. **B**. New Zealand land area 3 million years ago, from Trewick and Bland (2011). Light grey represents areas of low-lying land and marginal-marine deposition. **C.** Approximate land area 3Ma for each of the Crosby regions.

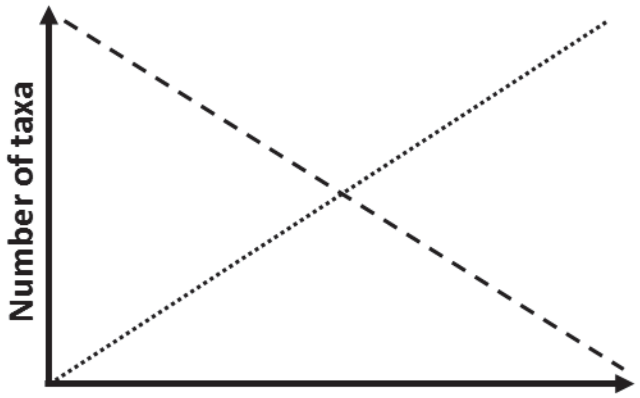

Figure 1.5. If most invertebrate taxa in New Zealand are widespread then few taxa will be restricted to a small number of regions (dotted line). If range restriction is common then few taxa will be found in a large number of regions (dashed line), This relationship would also be expected if sampling effort for each taxon was poor.

Table 1.1. Three datasets were used in the analysis of microendemism in New Zealand. This included two well-sampled families (sand flies (Simuliidae) and wolf spiders (Lycosidae)) and a dataset of 2322 invertebrate taxa found only on North and South Islands (and some close offshore islands).

Dataset	Simuliidae (sand flies)				Lycosidae (wolf spiders)			All species			
Region	Number of sites	Endemism score	Total taxa	Endemic taxa	Endemism score	Total taxa	Endemic taxa	Total taxa	Endemism score	Endemic taxa	Well-sampled endemics
AK - Auckland	25	0.00	1	0	0	2	0	658	0.11	68	10
BP - Bay of Plenty	20	0.00	1	0	0.25	4	1	450	0.05	22	3
BR - Buller	30	0.00	4	0	0.01	4	0	566	0.07	36	8
CL - Coromandel	14	0.00	1	0	0	3	0	362	0.03	9	1
CO - Central Otago	36	0.00	2	0	0	8	0	287	0.1	28	4
DN - Dunedin	13	0.00	2	0	0.01	2	0	239	0.07	15	2
FD - Fiordland	26	0.20	5	1	0	3	0	344	0.16	54	22
GB - Gisborne	12	0.00	1	0	0	0	0	209	0.03	5	1
HB - Hawkes Bay	18	0.00	1	0	0	1	0	213	0.04	7	0
KA - Kaikoura	14	0.00	2	0	0.01	1	0	173	0.04	7	1
MB - Marlborough	18	0.00	3	0	0.01	2	0	310	0.06	16	2
MC - Mid Canterbury	46	0.17	6	1	0	9	0	490	0.11	54	8
MK - Mackenzie	14	0.00	4	0	0	7	0	195	0.06	10	1
NC - North Canterbury	32	0.00	5	0	0	6	0	279	0.03	6	1
ND - Northland	48	0.00	0	0	0.34	3	1	596	0.18	108	33
NN - Nelson	61	0.00	3	0	0	5	0	763	0.14	101	26
OL - Otago Lakes	11	0.00	4	0	0	4	0	287	0.07	18	0
RI - Rangitikei	14	0.01	2	0	0	1	0	215	0.02	4	0
SC - South Canterbury	16	0.01	3	0	0.01	5	0	140	0	0	0
SD - Marlborough Sounds	7	0.00	3	0	0	0	0	287	0.05	13	0
SL - Southland	32	0.00	2	0	0.01	3	0	262	0.08	21	3
TK - Taranaki	18	0.00	1	0	0	2	0	308	0.04	11	5
TO - Taupo	37	0.01	2	0	0	2	0	444	0.04	16	1
WA - Wairarapa	13	0.00	1	0	0	2	0	184	0.02	3	0
WD - Westland	31	0.15	7	1	0	3	0	414	0.09	35	10
WI - Wanganui	5	0.00	1	0	0	1	0	198	0.03	5	0
WN - Wellington	15	0.00	1	0	0	2	0	548	0.08	42	6
WO - Waikato	14	0.00	1	0	0	1	0	302	0.07	20	4

Methods

The geography, geology and climate of New Zealand

New Zealand is an archipelago of mountainous subtropical and temperate islands situated in the South Pacific Ocean. The islands have a total area of approximately 268,000km², which is about 14 times the size of New Caledonia (Neall and Trewick 2008), but only about half the size of Madagascar. The two main islands of New Zealand are orientated north-south and are longer than they are wide, stretching approximately 1500km from 34˚25’ in the north to 47˚20’ in the south. This latitudinal spread generates a pronounced climatic gradient across the archipelago. New Zealand is positioned at the active boundary between the Australian and Pacific tectonic plates that generates earthquakes, mountain building and volcanism. During the last five million years this activity has substantially shaped New Zealand and modified its topography with North and South Islands only acquiring their current configuration in the last half million years (Trewick and Bland 2011).

South Island is older and more mountainous than much of the North Island landscape. Its axial ranges along the Alpine Fault had formed by 4 million years ago and are unlikely to have changed significantly in altitude since that time (Trewick and Bland 2011). These ranges are steep and were glaciated during Pleistocene climate cycling. The axial ranges generate high orographic rainfall to the west where low altitude native forest is relatively intact. The rainshadow to the east of the island produces dry plains that are now, like most of North Island, largely modified pasture. Southern North Island had yet to emerge from the ocean about 3 million years ago (Fig. 1.4B) (Trewick and Bland 2011), while the northern area of North Island is older. The northernmost regions of North Island were at times reduced to a series of islands (Fleming 1979; Morgan-Richards 1997). The centre of North Island has experienced a series of large eruptions associated with the Taupo Volcanic Zone (TVZ) that have likely reduced local biological diversity. Cook Strait, the approximately 25km wide stretch of sea between North and South Islands, formed about 500,000 years ago, so before this time, landscape in what is now the Wellington region was probably connected to Marlborough, South Island. At times of low sea level during glacial periods, and certainly during the Last Glacial Maximum at the end of the Pleistocene, there was probably a land connection between modern North and South Islands.

Invertebrate diversity

Crosby regions (Crosby *et al.* 1998; Crosby *et al.* 1976) provide a reasonable working hypothesis for investigating microendemism in New Zealand invertebrates. The type of data reported in each FNZ edition varies but most have species presence/absence per Crosby region. In order to explore patterns of endemism in North and South Islands I only included invertebrates that are endemic to either North and/or South Islands. This excluded most offshore islands but some near-shore Islands (e.g. Kapiti) are included within a mainland Crosby region. For further details of which islands are included with each region see Crosby *et al.* (1998) and the LENZ layer 'NZ Area Codes for recording specimen localities'.

A total of 2322 species were used in the analysis derived from 63 FNZ editions (1-13; 15-18; 20-36; 39-59; 61-68) and encompasses 12 invertebrate orders and the superorder Acari. Formicidae (ants) were also included using data from Don (2007). All introduced species were excluded. The presence/absence in each region of species endemic to some part of the main islands was used as a measure of species diversity. Species diversity was thus defined as the number of different species recorded in a region, and the number of endemic species were those recorded in that region and nowhere else. An endemism score for each species was expressed as the inverse of the mean geographic distance between the regions in which it occurred, but species present in only a single region received a score of one. Mean geographic distance between the regions was calculated as the distance between the centres of each Crosby region following the approach of Taberlet *et al.* (2012). The endemism score for each region was calculated as the mean endemism among all species occurring in the region. Endemism score thus accounts for variation in overall species diversity among regions.

I tested to see whether the microendemic species were evenly distributed among regions using a chi-square test. The expected values were calculated as the total number of endemic species in the analysis divided equally among the number of regions (n=28). The observed values were the numbers of endemic species in each region. I repeated this test to take into account the effect of differences in area of each region by calculating the proportion of New Zealand surface area represented by each region and multiplying this with the total number of endemic species.

Sampling effort

A large number of species were recorded from a just one sampling site and so automatically classified as microendemics. To test whether the high proportion of endemic species in some regions resulted from low sampling effort, I excluded all taxa that were recorded from just one site, unless the overall sampling of the genus or family was extensive and provided evidence of nearby absence. I defined sites as locations that were ≥5km apart. This reduced the number of microendemic taxa within the sample from 734 to a conservative 152. The relationship between initial estimates of microendemism within each region and the number of well-sampled microendemics was investigated.

To further explore whether regional variation in endemism was evident when location sampling is abundant, I used two densely-sampled datasets: Simuliidae (Insecta: Diptera) (Craig *et al.* 2012) and Lycosidae (Arachnida: Araneae) (Vink 2002). I also investigated whether Simuliidae regional diversity and endemism were correlated with sampling effort.

Comparison of endemism with geography/environment

To investigate potential causes of variation in regional diversity and endemism I looked for relationships between diversity/endemism and geophysical and environmental parameters that may affect speciation or extinction rates, or promote sampling bias.

Mean annual temperature and mean annual solar radiation were included because these variables may affect mutation rate and thus speciation rate (Dowle *et al.* 2013). Monthly water balance ratio (the average of the monthly ratios of rainfall to potential evaporation) was used as a measure of water availablitiy since aridity may have effects on patterns of microendemism, as found in Madagascar (Wilmé *et al.* 2006). Measures of slope and elevation were included as proxies for habitat heterogeneity because areas with substantial topographic variation have a greater variety of habitats (Veech and Crist 2007). Slopes increase community complexity and areas with slopes tend to be more diverse (Wollenberg *et al.* 2008) and contain species with small ranges (Orme *et al.* 2006). These environmental descriptors were derived from the Land Environments of New Zealand (LENZ) database (Leathwick *et al.* 2002; Leathwick *et al.* 2003). Current land area and the mean and variance of each region for each variable was calculated in ArcMap from ArcGIS 10.1 (ESRI, Redlands, CA, USA) using Crosby regions (LENZ layer 'NZ Area Codes for recording specimen localities').

I also investigated the influence of current land area and inferred land area 3 million years ago (Ma). I chose approximate land area 3Ma rather than anything older because previous research has indicated that patterns of genetic and species diversity are more likely to be related to processes acting in the past few million years (McGlone *et al.* 2001; Trewick and Wallis 2001). Approximate land area 3Ma (Fig. 1.4C) was calculated for each region (Fig. 1.4B) excluding low-lying land and marginal-marine deposition (Trewick and Bland 2011). Only three regions had no change in size (OL, MK, CO). As a measure of habitat availability I included an estimate of current forest cover in each region and as a measure of the recent impact of human arrival I used prehuman forest cover, calculated from McGlone (1989). There is a global relationship between latitude and biodiversity (Dowle *et al.* 2013), so the influence of latitude on New Zealand diversity was also investigated. Latitude was taken at the centre of each Crosby region. As a proxy for the connectivity/isolation of each region I used the number of neighbouring regions sharing a boundary.

I used the relative human population size of each region and the presence of a university in the region (a binary variable) as a measure of sampling bias. This is because more people and more researchers within a region increase the chance of human contact with a particular species and thus increase the likelihood of collection and analysis. The relative population size of each region was calculated based on 2006 census data using the 'populated places' polygon (by Peter Scott, from Koordinates.com), which defines populated places as areas where densities of approximately 20 persons per hectare exist. The size of the total populated area was then divided by the total area of a region to give the relative population size.

I looked for correlations of diversity and endemism (number of endemic taxa and endemism score) with these environmental variables, then fitted quasi-poisson-distributed generalised linear models (GLM) using endemic taxa in R v3.1.3 (R Development Core Team 2010) to see which combination of variables best explained the number of endemic taxa present in each region. Many environmental variables had strong relationships with one another (Table 1.2), so they were not all included in the model although I tested many models substituting correlated variables for one another. The best model was decided by the Akaike information criterion (AIC), ANOVA and diagnostic plots, and did not include all uncorrelated variables.

Table 1.2. Across New Zealand, many environmental variables are significantly correlated with one another. Shown here are R values: Red cells indicate a strong relationship ($R^2 \geq 0.64$, $R \geq |0.80|$); orange cells indicate a moderate relationship ($R^2 = 0.36\text{-}0.63$, $R = |0.6\text{-}0.79|$); blue cells indicate a weak relationship ($R^2 = 0.14\text{-}0.35$, $R = |0.37\text{-}0.59|$); no colour indicates little or no relationship ($R^2 < 0.14$, $R < 0.37$).

		Radiation		Temperature		Water Balance		Latitude	Neighbours	University	Slope		Elevation		Population (as % of area)	Current forest area (%)	Prehuman forest area (%)	Land area
		Mean	Variance	Mean	Variance	Mean	Variance				Mean	Variance	Mean	Variance				Current
Radiation	Variance	-0.21																
Temperature	Mean	0.69	-0.54															
	Variance	-0.26	0.4	-0.76														
Water Balance	Mean	-0.48	0.25	-0.44	0.49													
	Variance	-0.54	0.29	-0.64	0.66	0.79												
Latitude		-0.86	0.39	-0.89	0.51	0.31	0.51											
Neighbours		-0.33	0.22	-0.44	0.37	0.3	0.34	0.28										
University		-0.01	-0.29	0.23	-0.17	-0.2	-0.11	-0.06	-0.13									
Slope	Mean	-0.16	0.38	-0.59	0.64	0.65	0.54	0.35	0.09	-0.38								
	Variance	-0.47	0.38	-0.75	0.82	0.74	0.88	0.56	0.3	-0.18	0.73							
Elevation	Mean	-0.31	0.56	-0.87	0.86	0.4	0.62	0.57	0.48	-0.34	0.65	0.76						
	Variance	-0.37	0.41	-0.78	0.98	0.53	0.73	0.58	0.39	-0.17	0.6	0.86	0.84					
Population (as % of area)		0.28	-0.29	0.46	-0.32	-0.21	-0.22	-0.37	-0.24	0.65	-0.37	-0.3	-0.43	-0.33				
Current forest area (%)		-0.08	0.18	-0.17	0.16	0.65	0.26	0.05	0.02	-0.21	0.69	0.41	0.13	0.15	-0.17			
Prehuman forest area (%)		0.34	-0.6	0.83	-0.92	-0.53	-0.72	-0.56	-0.45	0.26	-0.67	-0.82	-0.94	-0.91	0.34	-0.17		
Land area	Current	-0.39	0.31	-0.35	0.22	0.29	0.26	0.25	0.33	-0.16	-0.03	0.25	0.34	0.24	-0.34	-0.01	-0.28	
	3Ma	-0.25	0.31	-0.36	0.3	0.28	0.24	0.24	0.05	-0.15	0.23	0.27	0.38	0.31	-0.2	0.19	-0.36	0.67

Results

Microendemism in New Zealand

Microendemism in New Zealand was investigated using the presence or absence of 2322 invertebrate species in 28 regions of New Zealand. Species diversity varied among New Zealand regions (Fig. 1.6, Table 1.1) and this was positively correlated with endemism (number of endemic species) ($R^2=0.73$ $p<0.001$) (Fig. 1.7A). Nelson region (NN) had the highest species diversity (763) and South Canterbury (SC) had the fewest species (140) of which none were endemic to the region (Table 1.1). Less than 11% of species were found in 10 or more of the 28 regions. Most species (75%) were found in 5 or fewer regions and 734 species (32%) were found within a single region i.e. microendemics (Fig. 1.8). The microendemics were not evenly distributed among regions $\chi^2(27, n = 28) = 809.28$, $p < 0.0001$), even when corrected for area $\chi^2(27, n = 28) = 772.74$, $p < 0.0001$). Northland (ND) had the highest number of endemic species (108) followed by Nelson (101) (Fig. 1.6B). Together these two regions account for 28.5% of all of the endemic taxa but only 8.8% of the total land area of New Zealand. In contrast Hawke's Bay (HB), Gisborne (GB), Wanganui (WI), Rangitikei (RI), Wairarapa (WA), Kaikoura (KA), north Canterbury (NC) and south Canterbury (SC) accounted for 23% of total land area but only 5% of all endemic taxa.

Sampling effort

Variation in sampling effort might be responsible for some of the variation in endemism levels between regions and for the pattern of predominant range restriction. Therefore I first explored the effects of variation in sampling effort by looking at patterns of endemism in two intensely-sampled families of flies (Simuliidae) and spiders (Lycosidae). While many FNZ editions rely largely on museum collections and opportunistic sampling, these two studies used intensively sampled data. Each searched site provides independent presence/absence data for each species. Since these groups have been densely sampled country-wide it is less likely that diversity statistics are biased by sampling. For Simuliidae eleven species that are endemic to the main islands of New Zealand (i.e. North, South and close offshore islands) were included in the analysis. Eighteen Lycosidae species were analysed. For both families I found that most species were recorded from few regions while few species were widespread (Fig. 1.9). This matches the pattern of predominant range restriction seen in the larger invertebrate dataset (Fig. 1.8), although this dataset was too small to test for significant heterogeneity between regions.

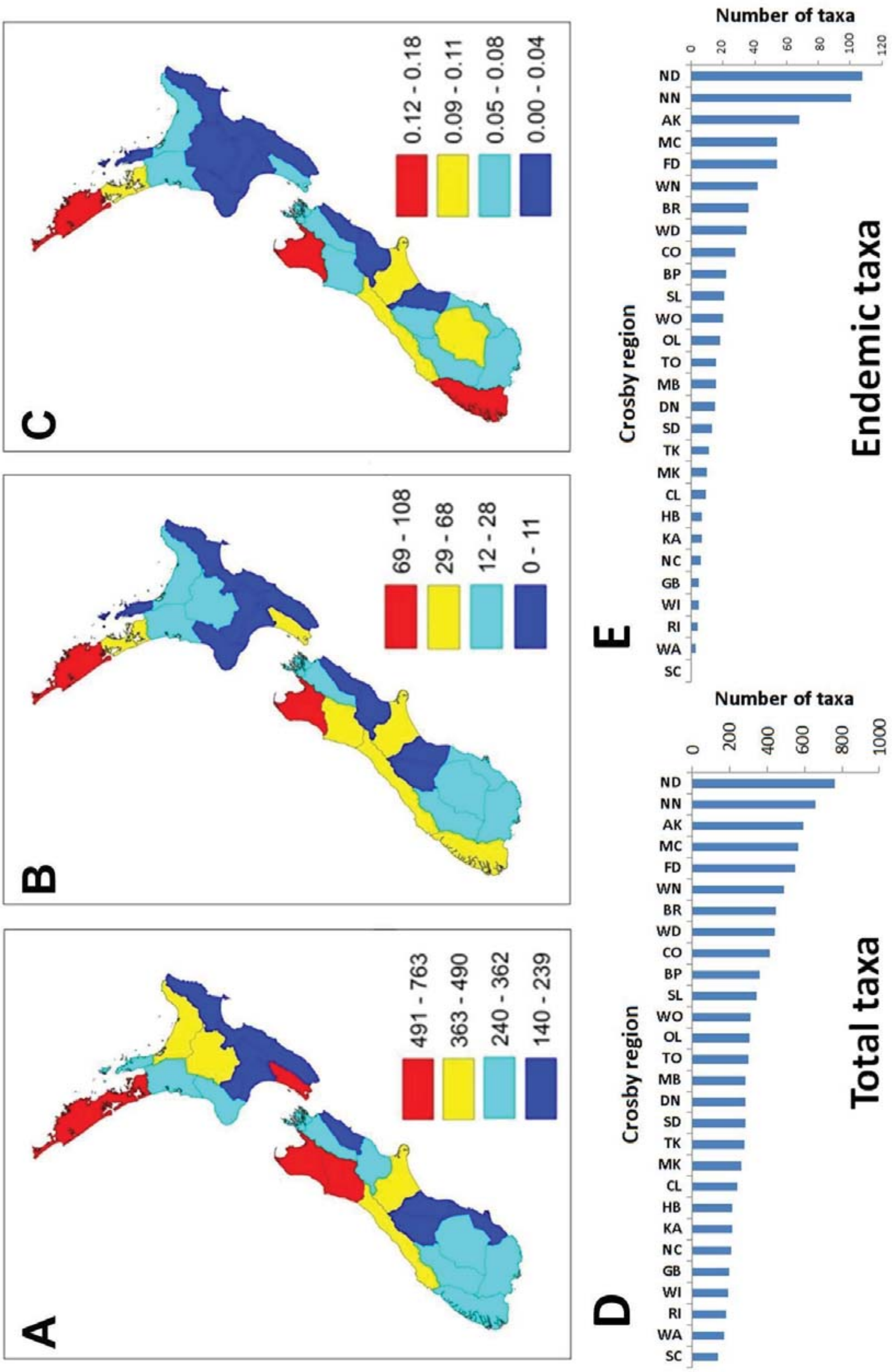

Figure 1.6. Endemism levels are not homogeneous among regions of New Zealand; some regions have particularly high endemism compared to others. **A.** Total invertebrate diversity out of a sample of 2322 taxa. **B.** Number of endemic invertebrate taxa per region. **C.** Endemism score for the region (average level of endemism for each taxon). **D**. Total diversity within each region. **E**. Total number of endemic taxa in each region.

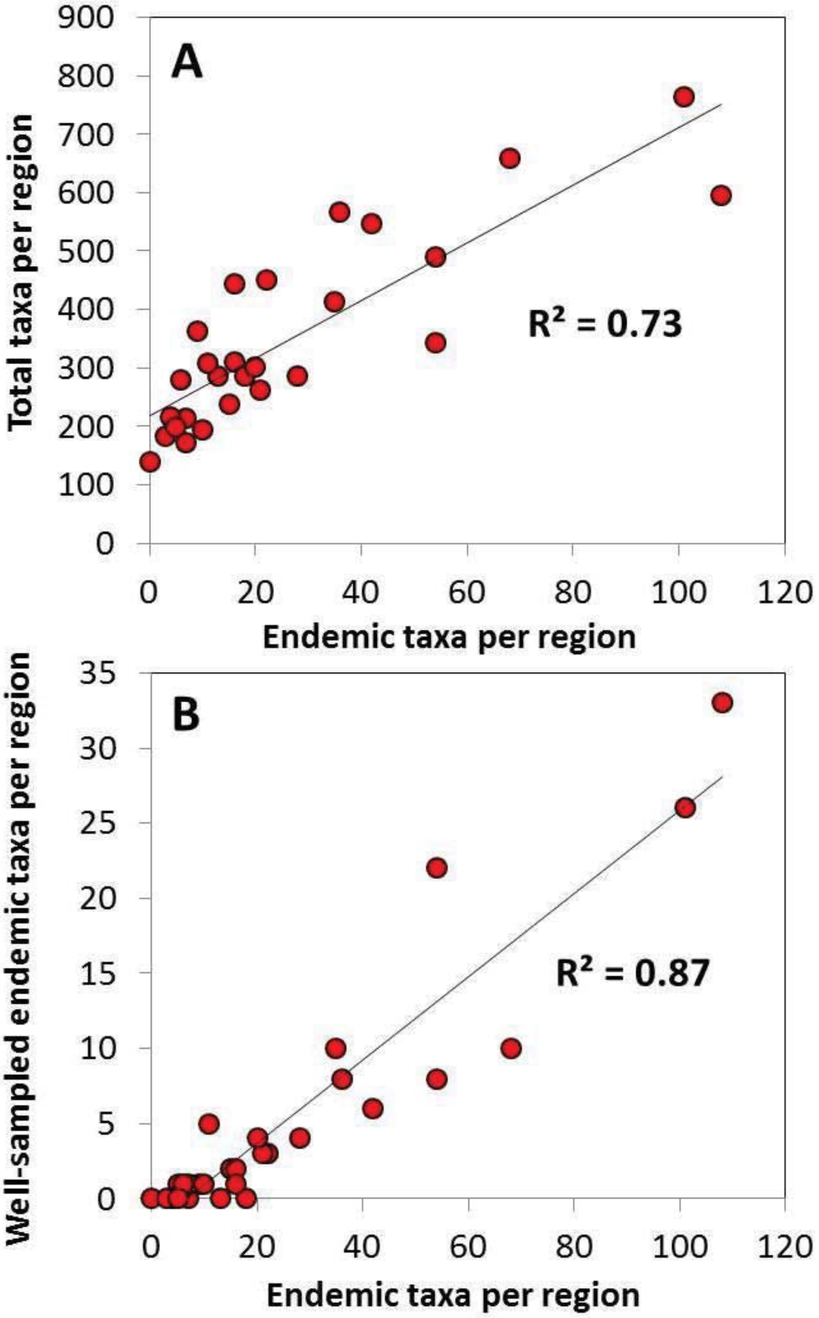

Figure 1.7. A. Microendemism and diversity are correlated in a sample of 2322 New Zealand invertebrates, 734 of which are microendemics. **B.** Well-sampled taxa (n=152) were strongly correlated with endemic taxa.

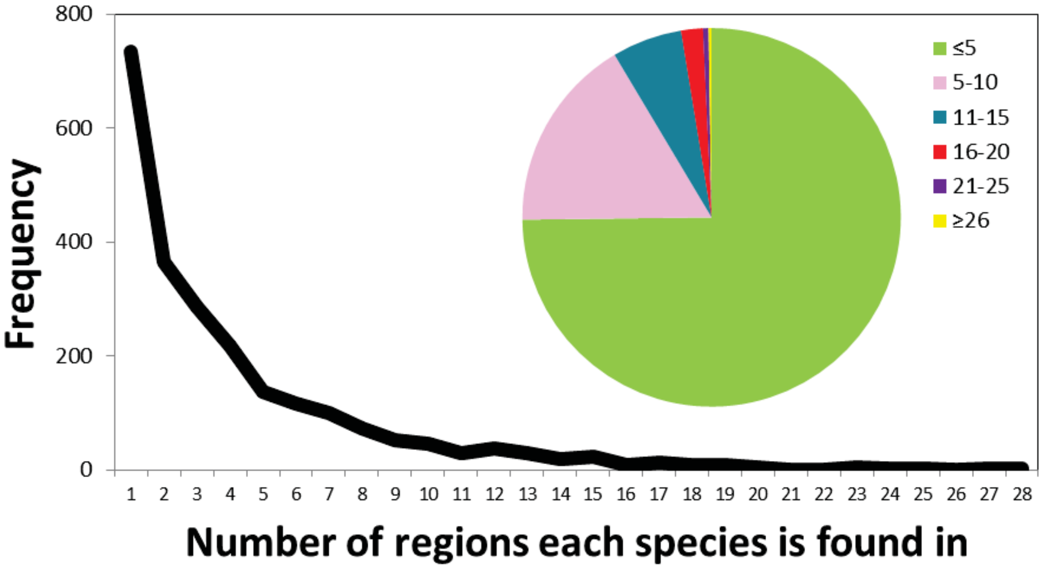

Figure 1.8. In a sample of 2322 New Zealand invertebrate species, most were reported from only few regions compared to a small number of widespread species. The pie chart shows that 75% of species are found in five or fewer of the twenty-eight entomological regions of the two main islands of New Zealand.

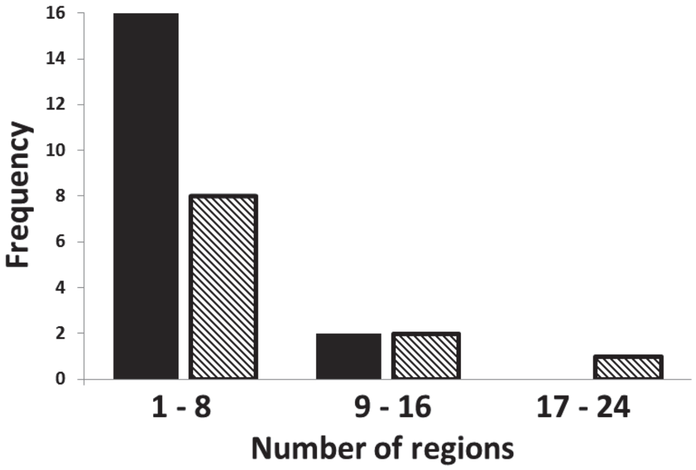

Figure 1.9. Most species of the well-sampled spider (Lycosidae (black) and sand fly (Simuliidae (striped)) families are found in few regions while a small number of species are widespread. None of the species included in the analysis were found in more than 24 of 28 regions.

I also explored how site density of a well-sampled dataset varied between regions and what affect this had on endemism and diversity. The per-region sampling of Simuliidae in terms of sampled localities was highest in Nelson with 61 sample sites and lowest in Wanganui with only five (mean 23 sites, median 18) (Fig. 1.10). An ideal expectation would be that sampling intensity was proportional to region area, and this is what I found. There was a significant positive relationship between land area and the number of collecting sites within a region ($R^2=0.35$, $p<0.001$). Although sampling effort (number of sites) within a region was significantly positively correlated (albeit weakly) with species diversity ($R^2=0.15$,$p=0.04$), there was no relationship between diversity and the size of a region (land area; $p>0.05$). Furthermore, sampling effort was correlated with neither endemic taxa ($p=0.10$) nor endemism score ($p=0.15$).

Figure 1.10. The distribution of Simuliidae (Sand flies: Diptera) sampling sites in New Zealand.

To further explore the effect of sampling effort on variation in endemism levels between regions I reanalysed the larger dataset of 2322 species. Most taxa (84%) that were found to be endemic to a single Crosby region were sampled from only a single site, therefore to explore whether the data were dominated by sampling effort I excluded taxa sampled from one site and that had poor overall sampling of the group. This reduced the number of microendemic taxa from 734 to 152 species with better sampling. I found that there was a strong relationship between the number of endemic taxa in each region and the number of well-sampled endemic taxa (R^2=0.87, $p \leq 0.001$; Fig. 1.7B), indicating that overall sampling is representative of actual endemism levels within regions.

Relationship between endemism and environmental variables

The relationship between endemism and the environment was tested using a set of environmental variables. There was no correlation between endemism or diversity and most of the environmental variables, with the only significant correlation (after Bonferroni correction) being between endemism score and land area 3 million years ago (R^2=0.34, p=0.001) (Fig. 1.11).

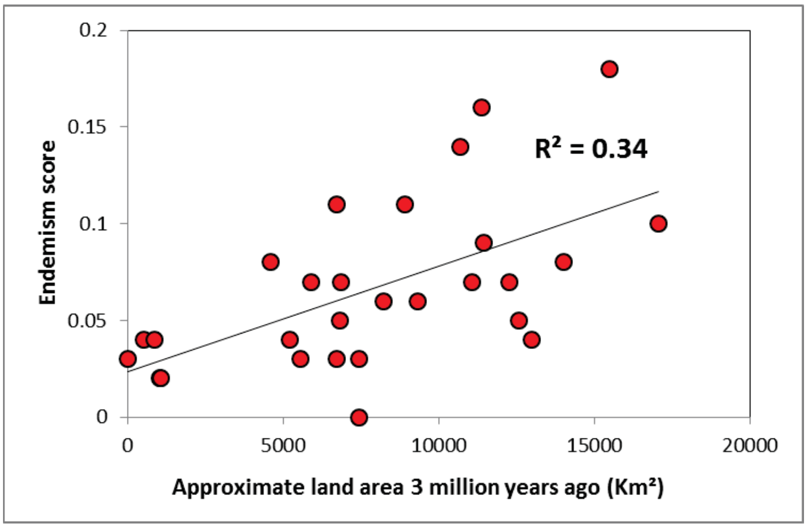

Figure 1.11. New Zealand microendemism score calculated from 2322 species was significantly correlated with land area 3 million years ago (R^2=0.34, p=0.001). Endemism score was the mean endemism among all species occurring in the region, calculated as the inverse of the mean geographic distance between the regions in which it occurred.

I developed a regression model to bring together the environmental variables to test which variables best explained the number of endemic taxa sampled from each region. Since 'endemic taxa' is count data, with some small values, I modelled it using a generalised linear model

(GLM) with a quasi-Poisson distribution that corrects for overdispersion. Many environmental variables were significantly correlated with one another (Table 1.2), so not all were included in initial models. Variables were removed by a stepwise search for the best model. No interactions were included as no significant interactions improved the fit of the model, judged by the AIC and by diagnostic plots. The total number of species (β =0.016, *p*<0.0001) and land area 3Ma (β =0.018, *p*<0.0001) had the largest significant positive impact on the number of endemic taxa followed by the presence of a university (β =0.01, *p*<0.0001) and mean monthly water balance ratio (β =0.017, *p*<0.0001) (Table 1.3). Endemic taxa decreased with the number of neighbouring regions (β =-0.01, *p*<0.0001).

Table 1.3. Regional variation in the number of endemic invertebrate taxa in New Zealand investigated with a quasi-Poisson generalized linear model (GLM). The best fitting explanatory variables were species diversity, past land area, water balance, the presence of a university and the number of neighbouring regions. All variables included in the model were significant predictors of endemism. β is the standardised coefficient indicating relative influence of each variable. For example, a change in one standard deviation of land area 3Ma has about twice the impact of the number of endemic taxa within a region than a one standard deviation change in water balance; t is the test statistic.

Explanatory variable	β	t	*p*
Total species	0.016	10.08	<0.0001
Land area 3Ma	0.018	7.41	<0.0001
University	0.010	5.64	<0.0001
Neighbours	-0.010	-5.60	<0.0001
Mean monthly water balance ratio	0.009	5.56	<0.0001

Discussion

The patterns

Sampling strategy can affect inferred patterns of diversity. For example, an intensely sampled site may artificially appear to be an endemism hotspot. A comparison of the dataset of endemic New Zealand invertebrate species to a subset of intensely-sampled endemic species showed that there was a strong relationship between them (Fig. 7B), suggesting that the total data do contribute meaningful data for analysing patterns of microendemism in New Zealand. Furthermore, microendemism in two intensely-sampled families of New Zealand invertebrates, Lycosidae and Simuliidae, provide by default, information on absences; the explicit reporting of the presence of particular species at a location indicates that other species were not found there. If endemism was an artefact of repeated sampling and analysis of specimens from particular regions, homogenous levels of endemism between regions in groups of well-sampled taxa would be expected. Within Lycosidae and Simuliidae, species distributions were heterogeneous with few widespread but many restricted species (Fig. 1.9). Likewise, within the dataset of 2322 taxa, widespread species were uncommon compared to range restricted species (Fig. 1.8).

This type of range restriction has been reported in other animals. For example, 27% of bird species worldwide have range sizes smaller than 50 000 km² which is approximately one fifth the area of New Zealand (Stattersfield *et al.* 1997). The reason why widespread species are uncommon may be that speciation occurs before a species expands its range beyond a certain size. In phylogeographic studies, widespread species are often used to make inferences about past events (Hewitt 1996, 1999; Taberlet *et al.* 1998), but since widespread species do not seem to be the norm and inferences made by studies of widespread species may be misleading (Trewick *et al.* 2011).

Ideally, regions of high endemism should be self-delimiting rather than based on predefined boundaries (Crother and Murray 2011). As this can only be achieved with fine-scale nationwide sampling over many taxa, predefined boundaries are better than post hoc analyses of few taxa. The application of regions as large as those used by Wardle (1963) could obscure patterns of endemism, so resolution to smaller scale patterns such as Crosby regions or one degree grid squares is worthwhile. In reality, however, spatial boundaries of areas of endemism must be expected to be dynamic and 'fuzzy' because rates of speciation, extinction and migration vary (Crother and Murray 2011). There is therefore little sense in trying to determine the exact position of boundaries on large islands (e.g. Rogers (1989) updated position of the 'Taupo Line'). Nevertheless, congruence revealed by the application of different boundaries may help to inform on and develop hypotheses about processes. Crosby regions were used in the present study due to the nature of the available data. I found that endemism levels are not homogeneous among regions of New Zealand. Rather some regions have particularly high endemism while others have few or no endemic taxa (Fig. 1.6). Unlike previous analyses, I found relatively low endemism levels in Kaikoura (KA) and the Coromandel (CL).

The processes

Land area can have a positive influence on rates of endemism due its influence on speciation rates and extinction rates which tend to be higher and lower respectively in bigger areas (Crampton *et al.* 2010; Foote *et al.* 2007; Kisel and Barraclough 2010). Therefore I predicted that endemism would be highest in regions that have had more land available for a longer period of time. Some types of invertebrate species have been shown to have average durations of about nine million years (Foote *et al.* 2007), while mammal species have been suggested to have average durations of only one million years (Martin 1993). These timespans are compatible with genetic analyses that suggest geologically recent, rather than ancient processes are important for determining the distribution of a species (e.g. Trewick and Wallis (2001), McGlone *et al.* (2001)). I found that of all the environmental variables tested, only land area 3Ma was correlated significantly with endemism score, and no variables were correlated with species diversity or number of endemic species. Land area 3Ma was also one of the strongest correlates ($\beta = 0.018$, $p < 0.0001$) in the generalised linear model of number of endemic taxa. Current land area was not correlated with endemism and did not improve the GLM, suggesting that, land availability in the recent past has had a greater effect on endemism levels than currently available land. The most notable effector of the relationship between land area 3Ma and endemism is the lack of land in southern North Island which was still submerged until about 1.5 million years ago (Trewick and Bland 2011), and where there are currently few endemic species. In this scenario, historical biogeographical events are not masked by ecogeographical processes; recent geological history, rather than ecology, has made this a region of low endemism.

Diversity and endemism are similarly influenced by the processes of speciation and extinction and therefore, diversity was one of the strongest significant predictors of endemism ($\beta = 0.016$, $p < 0.0001$). Diversity and endemism are, however, affected differently by range expansion; expansion between regions decreases endemism within a region but has no impact on diversity. I found that the number of neighbouring regions impacted negatively on endemism within a region ($\beta = -0.01$, $p < 0.0001$), reflecting the ease with which species can expand their ranges into adjacent regions, thus reducing endemism.

The number of current neighbouring regions is an estimate of current connectivity but it does not take into account past connectivity which was greater for some regions than others. For example, although the Nelson region (NN) currently has high numbers of endemic taxa and few neighbours, during the LGM there was a land connection between this region and Wanganui (WI) and Taranaki (TK). Range expansion also requires ecological compatibility, so although for example the West Coast has many neighbours, the abrupt habitat change across the Southern Alps may restrict the ranges of species.

Endemism may appear to vary between regions due to differences in detectability associated with sampling strategy, human population size, and the distribution of expert knowledge. Although there was no evidence for a relationship between human population size and endemism, the presence of a university was related to an increase in the number of endemic taxa within a region ($\beta = 0.01$, $p < 0.0001$). This is concordant with the finding that only 15% of endemic taxa from Auckland (AK) and mid Canterbury (MC) (regions with universities) were well-sampled compared with 41% in Fiordland and 31% in Northland (regions without universities).

Climate and habitat could theoretically influence endemism in many ways. Some climate factors allow for the retention of endemism by survival of endemics in situ while other factors may promote speciation and extinction. In Madagascar, episodes of aridity have likely shaped patterns of microendemism by causing the restriction of species to wet regions (Wilmé *et al.* 2006). I found that average monthly ratios of rainfall to potential evaporation (mean monthly water balance ratio) had a small significant positive impact on endemism ($\beta = 0.017$, $p < 0.0001$). Mean monthly water balance ratio, however, is correlated with many other environmental variables, including slope (Table 1.2). Slope may impact speciation rates because topographic variation results in a greater variety of habitats (Veech and Crist 2007) and more complex communities (Wollenberg *et al.* 2008) and may thus result in the exertion of differential selective pressures and high rates of speciation relative to extinction (species pump model; (Moritz *et al.* 2000; Rahbek and Graves 2001). However, mountainous areas were impacted by glaciation during the last glacial maximum that very probably resulted in extinction of many taxa from these regions (Trewick *et al.* 2000). The effects of climatic factors are complex and the mechanism by which moisture has a significant positive effect on endemism levels is not currently understood.

Although the distribution of plants and animals may have been strongly influenced in some places by glaciation and aridification during the last glacial maximum, I did not test the glacial refugium hypothesis. Pollen, macrofossil, geomorphic and beetle evidence indicate that the most extensive forests in New Zealand during LGM were in northern North Island (McGlone *et al.* 2010). Many other regions that could have been LGM refugia for forests are currently under the ocean and much palynological evidence is also submerged. In particular the coastal areas most likely to have supported forest have been lost due to subsequent sea level rise (Trewick and Bland 2011). Furthermore, glacial refugia for insects may have existed anywhere that glaciers did not because high density populations can persist in small areas. Open forest, scrub and grassland may all have supported insect diversity and may have been widespread (Shepherd *et al.* 2007). Northland is relatively flat and dry compared to other regions of high invertebrate endemism, so its endemism may be better explained by past land area or past forest cover, whereas other regions may be more strongly influenced by other factors.

Conclusions

Previous observations indicated that endemism levels vary between regions of New Zealand. I investigated this further using a rich invertebrate dataset and found that sampling effort has some influence on the data. Despite this, I found that most species are range restricted and regions do differ in endemism levels. These differences appear to be related to geologically recent land availability, connectivity between regions or intensity of environmental gradients. This suggests that high levels of microendemism in some regions are not simply a product of the accumulation of species over time, rather microendemism depends on the ability of a region to retain species. Regions with fewer neighbours have lower connectivity and thus lower migration rates, whereas those with many neighbours are less able to retain endemic taxa however how old they are. The absence of land in southern North Island until recent geological time shows that tectonic activity in New Zealand has a massive and youthful impact on endemism levels and indicates that, in this situation at least, ecology plays a minor role. In contrast, microendemism in older, stabler regions is more likely to be influenced by steep environmental gradients.

Appendix

Environmental variables for each Crosby region of New Zealand. Regional codes from Crosby *et al.* (1976) (see Table 1.1 for full regional names). Environment data calculated from Leathwick *et al.* (2002), Leathwick *et al.* (2003), Trewick and Bland (2011), McGlone (1989) and Koordinates.com.

Region	Land Area (Km²)		Radiation		Temperature		Water Balance		Latitude	Number of neighbouring regions	Forest (Km²)		University	Slope (˚)		Elevation (m)		Percentage of area populated
	Current	3Ma	Mean	Variance	Mean	Variance	Mean	Variance			Current	Prehuman		Mean	Variance	Mean	Variance	
AK	5648	6693	15.09	0.03	14.40	0.28	2.57	0.12	36.59	3	359	5648	1	5.95	32.38	79.19	5806	8.58
BP	13526	12550	14.92	0.04	12.15	1.75	3.75	0.68	37.96	4	5904	13056	0	13.44	123.64	358.75	57204	0.95
BR	11227	11072	13.45	0.18	8.76	4.88	7.73	5.52	42.22	4	8300	6817	0	18.96	157.60	646.40	205018	0.11
CL	2930	6721	15.06	0.03	13.75	0.88	3.52	0.27	36.92	4	805	2930	0	13.13	62.31	185.19	23083	1.34
CO	17066	17066	13.26	0.15	7.33	3.35	2.48	1.28	45.20	5	260	11864	0	11.78	83.20	751.06	139221	0.18
DN	6687	5872	12.53	0.06	9.45	0.69	1.99	0.27	45.52	4	348	6687	1	7.84	49.70	231.41	29715	1.08
FD	13244	11361	12.18	0.07	7.28	3.45	11.90	26.59	45.30	3	9716	9128	0	24.96	230.07	606.99	162027	0.03
GB	10699	7435	14.80	0.03	12.29	2.33	3.58	1.23	38.44	3	1244	9990	0	13.67	56.69	372.76	75080	0.21
HB	7093	520	14.50	0.13	12.18	1.85	2.57	0.48	39.75	4	410	6554	0	9.07	61.26	280.60	69143	0.85
KA	5787	5203	14.65	0.04	9.19	6.41	2.62	0.95	42.19	2	601	4147	0	19.34	119.97	671.01	233179	0.06
MB	7568	9300	14.84	0.08	8.26	7.09	3.42	2.00	41.89	5	2324	3339	0	21.46	127.11	841.73	245739	0.25
MC	12534	8910	13.78	0.05	9.27	6.61	3.52	14.17	43.56	3	817	8876	1	10.31	174.38	539.55	286684	1.51
MK	8227	8227	13.80	0.14	6.76	6.14	5.34	28.66	44.05	5	164	3260	0	16.48	200.25	1049.31	267059	0.06
NC	8876	5534	13.96	0.08	9.15	4.98	3.55	8.89	42.99	5	2107	6080	0	14.10	150.01	578.45	200866	0.27
ND	12294	15484	14.96	0.03	14.78	0.49	2.79	0.29	35.40	1	715	12294	0	7.44	40.79	118.34	12644	0.47
NN	11061	10673	14.58	0.28	9.64	3.94	5.98	5.13	41.18	3	6314	7499	0	19.41	125.11	550.36	143286	0.42
OL	12241	12241	13.34	0.20	6.65	5.48	6.33	17.03	44.84	5	2533	4816	0	21.91	174.00	944.65	219345	0.18
RI	8815	1028	14.21	0.05	10.63	1.91	3.21	0.66	39.72	6	2704	7787	0	14.83	79.47	499.95	67104	0.13
SC	7836	7442	13.18	0.08	8.84	4.07	2.61	7.55	44.13	5	197	5727	0	11.25	130.88	539.08	227308	0.35
SD	1920	6828	14.78	0.04	11.41	0.99	4.21	0.96	41.05	2	1738	1920	0	22.02	75.41	251.15	37724	0.31
SL	14344	14001	12.41	0.06	9.34	0.82	3.28	0.64	46.05	4	1886	14191	0	5.57	49.91	209.52	40106	0.40
TK	8769	846	14.42	0.05	12.31	0.93	4.18	1.47	39.10	4	3143	8719	0	10.77	75.38	232.01	25844	0.59
TO	15531	12984	14.39	0.06	10.15	2.96	3.70	1.14	38.91	6	4586	13035	0	9.41	76.68	658.44	87634	0.27
WA	8135	1052	13.95	0.02	11.88	0.61	3.07	0.27	40.83	3	307	8135	0	10.12	55.09	212.54	16961	0.38
WD	12619	11419	12.73	0.07	8.15	7.99	15.50	31.62	43.56	7	8089	5838	0	21.70	224.05	697.93	353119	0.04
WI	4846	0	14.35	0.04	12.42	0.39	2.27	0.11	40.06	3	98	4846	0	6.33	62.56	138.60	12856	1.62
WN	4675	4596	14.01	0.01	10.94	2.70	4.45	2.68	40.87	3	1942	4260	1	14.97	120.93	327.88	76643	4.19
WO	11576	6846	14.60	0.04	13.35	0.47	3.05	0.54	37.92	5	1866	11576	1	6.00	44.22	125.94	13500	1.09

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Chapter 2. Nuclear marker development

Introduction

The insect mitochondrial genome is small (~15-18 kb) and haploid, and many mitochondrial genomes have been sequenced. As a result, the function and location of its genes are relatively well-known, and organism-specific primers can readily be designed for mtDNA regions. There are only a few difficulties associated with amplifying and sequencing mitochondrial genomes. One potential problem is the copying of parts of the mitochondrial genome into the nuclear genome resulting in Numts (nuclear mitochondrial pseudogenes (Richly and Leister 2004)). Numts are common in insects and have been reported in *Hemiandrus* (Chappell *et al.* 2012). They are readily, through inconsistently, amplified with mtDNA-designed primers during a polymerase chain reaction (PCR) along with or instead of mtDNA but they can be distinguished from mtDNA by the presence of frameshifts, unexpected stop codons, and abnormal basal placement of nuclear sequences within phylogenetic trees compared to other sequences from the same population (Bensasson *et al.* 2001). Despite this and other problems (e.g. heteroplasmy), mtDNA has been useful for resolving phylogenetic relationships at many taxonomic levels (Simon *et al.* 1994). The relatively high rate of molecular evolution of the mtDNA genome and the fact that it exists as many copies per cell make this genome especially useful for analysis of species diversity. A mitochondrial phylogeny tends to be the starting point of many phylogeographic investigations (Avise *et al.* 1987).

Since mtDNA genes are generally inherited as a linked unit, they represent only a single locus no matter how much of the mtDNA genome is sequenced. To account for the coalescent process, multiple loci are needed (Hudson 1990), and because nuclear genomes undergo recombination they consist of many independent loci. The animal nuclear genome is, however, large, of more complex organisation compared to the mitochondrial genome and fewer whole genomes have been sequenced. Much of the DNA in the nuclear genome exists as multiple copies, e.g. telomeres, microsatellites, transposable elements, the ribosomal cassette and histone genes. Developing taxon-specific nuclear sequence markers for non-model organisms is therefore more challenging.

Next-generation sequencing (NGS) technology now enables the rapid and anonymous sequencing of large parts of nuclear genomes in non-model organisms, allowing for rapid intensive data collection from a single run (Schuster 2007). When a fraction of the genome is sequenced at random, high-copy sequences are most likely to be amplified. The greater depth of coverage of high copy-number sequences allows the separation of high-copy regions such as mtDNA from lower-copy regions. Using NGS data, I aimed to develop primers for low or single-copy nuclear markers that could be used for phylogenetic analysis to help elucidate species boundaries in ground wētā.

Evolutionary relationships are most easily inferred from a single-copy nuclear locus inherited in a vertical Mendelian fashion. The alleles should have ample nucleotide variation between biological units of interest and orthologous loci should amplify using the same primers for all taxa in the study. The variation needs to be sufficient for phylogenetic analysis at the required level but not so much that signal saturation becomes a problem (Philippe *et al.* 2011). However, nuclear sequence markers might still be useful even if they are not single-copy, and multiple-copy loci are frequently employed in phylogenetic analyses (e.g.histone and ribosomal genes). A commonly used multi-copy locus is the internal spacer (ITS) region that is a noncoding part of the transcriptional unit located between the genes for the ribosomal subunits. The popularity of ITS as a molecular marker is largely due to its rapid rate of evolution providing genetic variation even when taxa are closely related. In animals, this region exists in hundreds of copies; approximately 500 copies in humans (Sakai *et al.* 1995). Identity between copies is maintained by concerted evolution, but this process may be incomplete resulting in paralagous ITS loci and so it is not ideal for phylogenetic analyses (Alvarez and Wendel 2003). Feliner and Rossello (2007), however, countered that ITS could still be useful because the problem of orthology will still be present in low-copy nuclear genes. Rather, they advised that it be used with caution and in conjunction with other loci. This demonstrates that when choosing markers a trade-off is frequently required by the investigator. The mechanism of evolution and transmission of a marker can influence analysis of evolutionary relationships but studies of host-parasite co-phylogeny has demonstrated that close associations on the time scale of interest can provide excellent evidence for phylogenetic studies (e.g. Morelli and Spicer 2007; Maneesakorn *et al.* 2011). Thus a balance between information content and observance of phylogenetic assumptions are required when selecting markers for inferring evolutionary relationships.

Methods

DNA extraction & sequencing

Genomic DNA was extracted from four Anostostomatidae (two ground wētā species (*Hemiandrus pallitarsis* and *H. maculifrons*) and two outgroup species (*Hemideina crassidens*, *Deinacrida connectens*)) species using a CTAB extraction. The extraction of DNA from *Hemiandrus pallitarsis* used three individuals from the same location. The genomic DNA was subjected to high throughput sequencing using Illumina High-Seq 2000 (BGI, Hong Kong) to generate a minimum of 1GB of data per taxon. Some species were placed in a mixed run with a variety of other vertebrate and invertebrate species, the rest were indexed and run individually.

Primer design

The high quality data (quantified using FastQC and SolexaQA (Andrews 2011; Cox *et al.* 2010)) was de novo assembled using Velvet v1.1 and ABySS v1.3.3 (Simpson *et al.* 2009; Zerbino and Birney 2008). This generated mtDNA assemblies for each taxon and additional nuclear DNA consisting of between 757 and 1,584,450 contigs that ranged in size from 100 to 9,425 bases. The *H. pallitarsis* contigs were mapped to those of *H. maculifrons* using Geneious v6.1.6 (Kearse *et al.* 2012) resulting in 221 alignments representing potential homologous loci (Fig. 2.1). The alignments were screened for length, polymorphic sites, multiple copies and primer binding sites. Primers for PCR were designed for nine suitable alignments using Primer3 (Untergasser *et al.* 2012) implemented in Geneious.

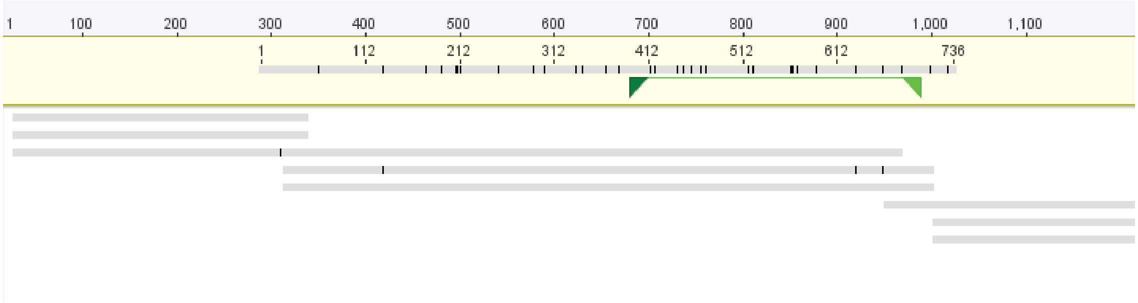

Figure 2.1. *Hemiandrus pallitarsis* contigs (bottom) mapped to an *H. maculifrons* reference contig (top). Primers (green triangles) were designed based on this consensus sequence to enable targetted amplification using polymerase chain reaction.

Locus identification

A nucleotide BLAST search (Basic Local Alignment Search Tool) v2.2.26 (NCBI) was carried out on each putative locus but no matches were found. An amino acid translation of the original alignment using the standard genetic code and all potential reading frames showed that the loci contained open reading frames (ORF) consisting of a minimum of 993bp. An open reading frame is a continuous stretch of DNA sequence that codes for a protein or peptide, and usually contains a start and stop codon. This translation was protein Blasted using default settings to find similar sequences in a public data base (GenBank).

Presence in outgroup species

I searched for each of the loci in NGS datasets from two outgroup species (Giant wētā *Deinacrida connectens* and tree wētā *Hemideina crassidens*). This was done by mapping outgroup NGS sequence contigs to the *Hemiandrus* contigs, and then Blasting the alignments in Geneious v6.1.6 using the longest ORF from the original alignment for each locus.

Primer trial

Novel primer pairs were subjected to PCR using template DNA from 23 individuals (Table 2.1) comprising eight *Hemiandrus* species for which mtDNA sequence data was already available (Pratt *et al.* 2008). Species were included to represent known phylogenetic diversity. PCR was performed under the following conditions: 94°C for 2 min then 35 cycles of 94°C for 30s, annealing at 56-59°C for 30s, and extending at 72°C for 30s; 72°C for 8 min. Amplification products that were visualised by gel electrophoresis as a single band were sequenced using traditional methods after being subjected to Shrimp Alkaline Phosphotase⁄Exonuclease I digestion (USB Corp.) to remove unincorporated primers. Cycle sequencing with the reverse PCR primers used Bigdye chemistry (PE) followed the manufacturer’s protocols, with automated reading on an ABI3730.

Table 2.1. Number of individuals, variable sites and alleles for each *Hemiandrus* species sequenced at each of the three nuclear loci. For specimen details, see appendix. **Hemiandrus maculifrons* has high morphological and genetic diversity (see chapter 3), but all individuals included here were the same morphospecies. **Three of these individuals were combined in one sample.

Clade	species	Nu2 (n=24)			Nu3 (n=15)			Nu13 (n=17)		
		# individuals	# variable sites	# alleles	# individuals	# variable sites	# alleles	# individuals	# variable sites	# alleles
1	*maculifrons**	9	3	4	4	1	2	7	2	4
	“alius”	5	0	1	4	1	2	3	0	1
2	*focalis*	2	1	2	1	0	1	1	0	1
	electra	2	0	1	NA	NA	NA	NA	NA	NA
	maia	NA	NA	NA	1	0	1	1	0	1
	pallitarsis	5**	0	1	3**	0	1	4**	0	2
	“timaru”	1	0	1	NA	NA	NA	1	0	1
	“vicinus”	NA	NA	NA	2	0	1	NA	NA	NA

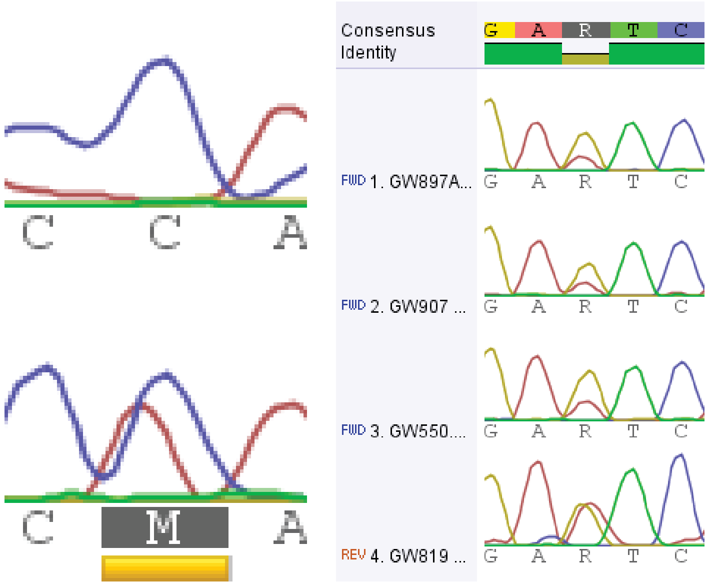

Figure 2.2. Left: Some double peaks were simply sequencing artefacts which disappeared with reamplification and sequencing with the forward primer. Right: At some positions, all individuals of the same species had the same double peaks. The ambiguity was retained in all individuals when sequenced in both directions.

Analysis of sequences and alignments

PCR products from three of the primer pairs tested consistently produced a single fragment and provided high quality DNA sequences. These sequences were edited and aligned using Geneious v6.1.6. Each sequence was inspected for ambiguous sites and those that contained ambiguities were re-amplified and sequenced in the opposite direction (using the alternative PCR primer) to check that ambiguities were not just sequencing artefacts (Fig. 2.2).

Some PCR fragments gave sequences expected of a heterozygous locus. Most putative heterozygous individuals provided sequences that contained only a single ambiguous site and so their two alleles could be resolved. Only *H. maculifrons* individuals had sequences with more than a single variable site. Since next-generation sequencing is a single-molecule sequencing method it automatically resolves the allelic phases of heterozygotes (Camargo and Sites 2013), so *H. maculifrons* haplotypes were resolved by comparison with the original alignment from which primers were designed. To visualise relationships between alleles, median joining networks (Bandelt *et al.* 1999) were constructed for each locus using Population Analysis with Reticulate Trees (PopART; otago.ac.nz).

Phylogenetic analysis

Phylogenetic relationships were inferred using the three loci independently. To determine the most appropriate model of DNA evolution, log-likelihood scores were calculated for models and compared under the Akaike information criterion (AIC) test in jModelTest v2.1.3 (Darriba *et al.* 2012) (Table 2.2). Bayesian phylogenetic analyses were conducted in BEAST v1.8.0 (Drummond *et al.* 2012) using a coalescent tree prior. An uncorrelated lognormal (UCLN) model of rate variation was assumed for each partition. Two independent runs were executed in BEAST v1.8.0 with each run consisting of 50 million generations sampling every 5,000 generations. Traces were checked for convergence in TRACER v1.5.0 (Rambaut and Drummond 2009). The first 10 million generations were discarded as burnin. Resulting tree files from each run were combined using the program LOGCOMBINER v1.8.0 (http://beast.bio.ed.ac.uk/LogCombiner). Pooled post-burn-in effective sample sizes for all parameters were >500, indicating that the pooled log file accurately represented the posterior distribution (Kuhner 2009).

For comparisons of the concordance of interspecific relationships between loci, a Bayesian mitochondrial DNA (COI) phylogeny was constructed for *Hemiandrus* species used in the trial of nuclear primers. The tree was rooted with homologous mtDNA sequences representing

Hemideina crassidens (tree wētā) *and Deinacrida connectens* (giant wētā) and inferred under a general time reversible (GTR) model including gamma and a proportion of invariant sites using Mr Bayes, implemented in Geneious. Genetic distances between species were calculated as pairwise uncorrected genetic distances.

Table 2.2. Length of longest open reading frame (ORF), length of PCR fragment and number of parsimony informative sites for each locus. Only a single locus (NU13) was found in the scree and the tree wētā, but part of NU2 was found in the giant wētā. Conserved domains associated with transposable elements were detected in each of the three loci and each locus protein-blasted to an insect protein or reverse transcriptase. The Expect value (E value) indicates the number of different alignments with scores ≥S (the sum of substitution and gap scores) that is expected to occur in a database search by chance. The lower the E value, the more significant the score and the alignment.

Locus	Length of longest ORF in original alignment (bp)	Length of PCR fragment	Parsimony informative sites	Present in out-group	Blastp results				Substitution model	Anneal temp
					Closest match	E value	Similarity (positive substitutions)	Conserved domains detected		
NU2	993	247 bp	15 (6.1%)	Partial (giant weta)	Hypothetical protein-butterfly (*Danaus plexippus*)	2e-37	54%	Endonuclease-reverse transcriptase	TRNef	56°C
NU3	1068	228 bp	21 (9.2%)	No	Endonuclease-reverse transcriptase-silkworm (*Bombyx mori*)	7e-104	64%	Reverse transcriptase	GTR	56°C
NU13	2607	194 bp	25 (12.9%) (excluding outgroup)	Yes (giant and tree weta)	Putative reverse transcriptase-fly (*Drosophila simulans*)	0.0	63%	Endonuclease-reverse transcriptase, reverse transcriptase	HKY+I	59°C

Results

Three putative nuclear loci (NU2, NU3, NU13) were reliably amplified with novel primers in multiple individuals, were visualised as a single band and were able to be sequenced (Table 2.2). Two outgroup species were also searched for these loci to determine whether the loci fulfil the assumption required of a phylogenetic marker of vertical transfer. NU13 was found in the nuclear contigs of giant and tree wētā. Only part of NU2 was found (Fig. 2.3). No significant match to NU3 was found in the outgroup species.

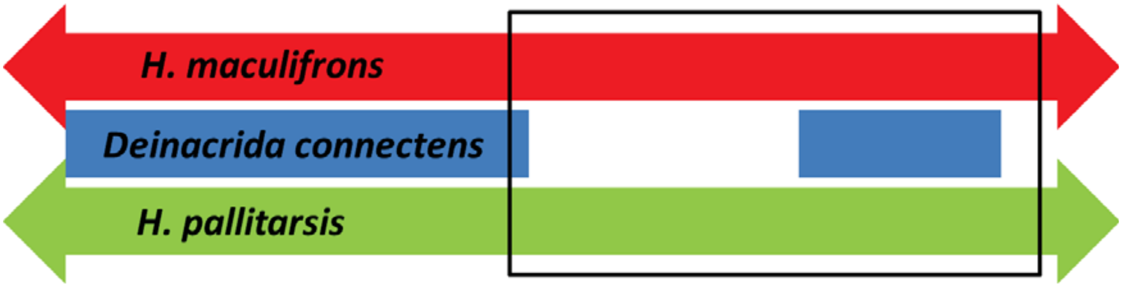

Figure 2.3. The locus NU2 was found in the NGS data of both *Hemiandrus maculifrons* and *H. pallitarsis*. Only part of NU2 was located in an outgroup species, *Deinacrida connectens*. The box indicates the region amplified by primers, while sequence outside the box represents the adjacent sequence.

The regions amplified did not have identical DNA sequences suggesting the markers might be suitable for phylogenetic analysis of *Hemiandrus*. The fragment amplified at locus NU2 was 247 bp and consisted of 15 (6.1%) parsimony informative sites (Table 2.2). The fragment amplified at locus NU3 was 228 bp and consisted of 21 (9.2%) parsimony informative sites. The fragment amplified at locus NU13 was 194 bp and consisted of 25 (12.9%) parsimony informative sites within ground wētā, but when outgroup species were included (tree and giant wētā) consisted of 42 (21.6%) parsimony informative sites.

Blast searches of translated sequences of the longest ORF in the original alignment of each of the three loci showed similarity to proteins from other insects that contain conserved reverse transcriptase domains (Table 2.2). These are domains present in retrotransposons, a class of transposable element (TE). The full length of NU3 (356 amino acids) aligned to a silkworm (*Bombyx mori*) endonuclease-reverse transcriptase, and NU13 (869 amino acids) aligned along nearly its full length (97%) to a *Drosophila simulans* (putative) reverse transcriptase. However, for NU2 (331 amino acids), only 80% of its length was aligned to a monarch butterfly (*Danaus plexippus*) hypothetical protein containing an endonuclease-reverse transcriptase domain. Each NGS contig sequence was too short to determine whether the region was transcriptionally active. The fact that for NU2 the matching region did not extend along the full length of the ORF suggests that either the TEs of wētā and butterflies are highly diverged (having shared a common ancestor a long time ago) or that they have been rendered inactive (in one or both genomes).

The chromatograms of the DNA sequences showed that some contained ambiguities, but very few ambiguities remained when loci were sequenced from both directions suggesting that most were sequencing artifacts. The presence of double peaks that were not sequencing artifacts suggested the presence of heterozygosity, as expected for a nuclear locus in a diploid animal. However I found that within some species, all individuals were apparently heterozygous at the same site (fixed heterozygosity), with no homozygous individuals detected (Fig. 2.2). The likely explanation for this is that more than a single allele or gene locus was amplified, although sample sizes per species were small (n < 10). So apparent high heterozygosity could be a sampling effect and the finding that for each individual a maximum of two alleles were present suggests that only two copies were amplified. The extraction of *H. pallitarsis* for NGS sequencing consisted of three individuals from the same location but only two alleles were present in this NGS data, and only two were present when more *H. pallitarsis* individuals were amplified and sequenced (NU2, NU13) (Table 2.1).

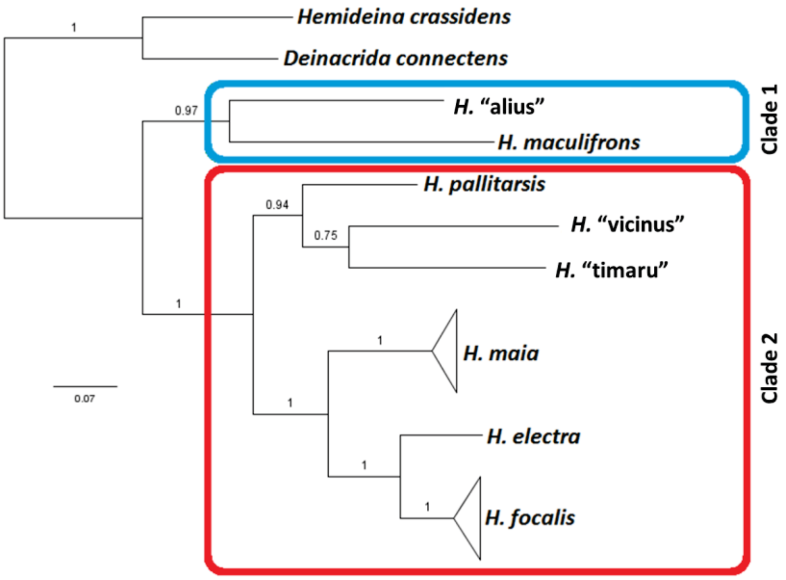

Figure 2.4. Bayesian mitochondrial DNA (COI) phylogeny constructed using *Hemiandrus* species from two clades (Pratt *et al.* 2008). These species were used in the investigation of nuclear loci for phylogenetic analysis. The tree was rooted with *Hemideina crassidens* (tree wētā) *and Deinacrida connectens* (giant wētā). Branch labels indicate posterior probabilities.

To assess the transmission of the three putative-loci I looked for concordance of phylogenetic signal in two ways. The mtDNA phylogeny of *Hemiandrus* rooted with giant and tree wētā showed there were two main clades within the genus: clade 1 and clade 2 (Fig. 2.4). All three nuclear loci returned topologies consistent with these two clades (Fig. 2.5, 2.6). NU13, which was also found in both outgroup species, supported the grouping of tree and giant wētā. There was also concordance between mtDNA and the three nuclear loci in their interspecific genetic distances (Fig. 2.7). NU2 and NU13 uncorrected pairwise genetic distances were strongly positively correlated with mtDNA distances (NU2 R^2=0.56; NU13 R^2=0.71). NU3 distances were also significantly correlated with mtDNA distances, although the relationship was weak (R^2=0.21). For NU2 and NU13 there was no overlap between within clade distances and among clade distances, but for NU3 some within clade distances exceeded some between clade distances. Some overlap of intra- and interclade distances is, however, also present within mtDNA. The general concordance between mtDNA and the three putative nuclear markers in terms of both tree shape and genetic distances suggests that the nuclear markers are evolving in a similar manner to mtDNA and are likely being transferred vertically among the specimens analysed.

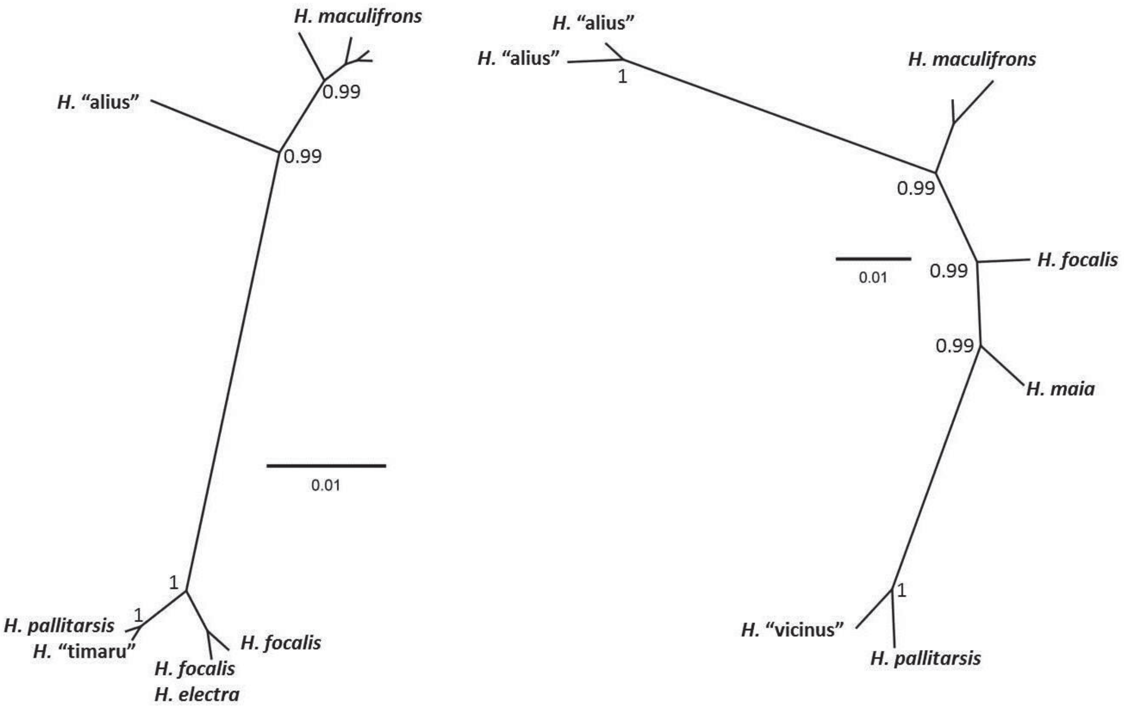

Figure 2.5. Unrooted phylogenies of NU2 (left) and NU3 (right) alleles.

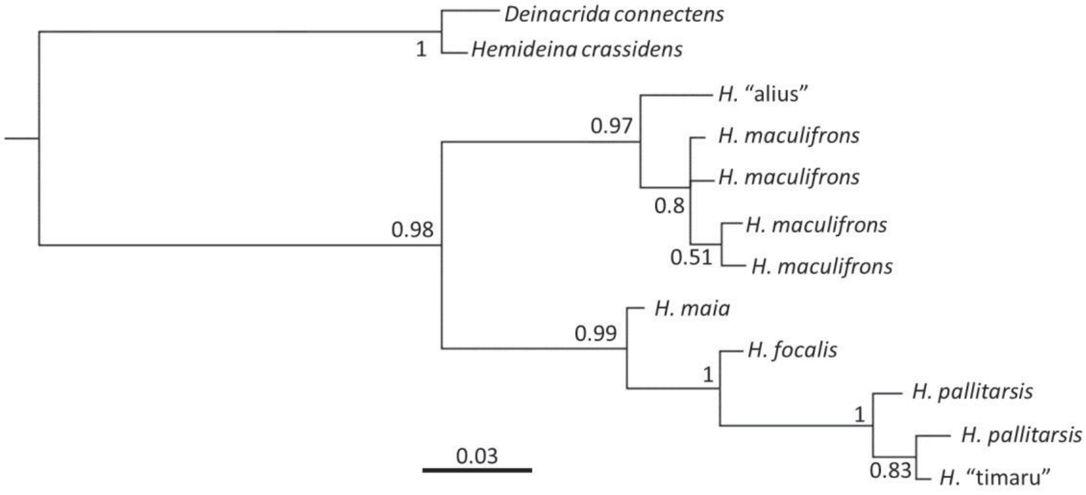

Figure 2.6. Phylogeny of NU13 alleles rooted with tree and giant wētā.

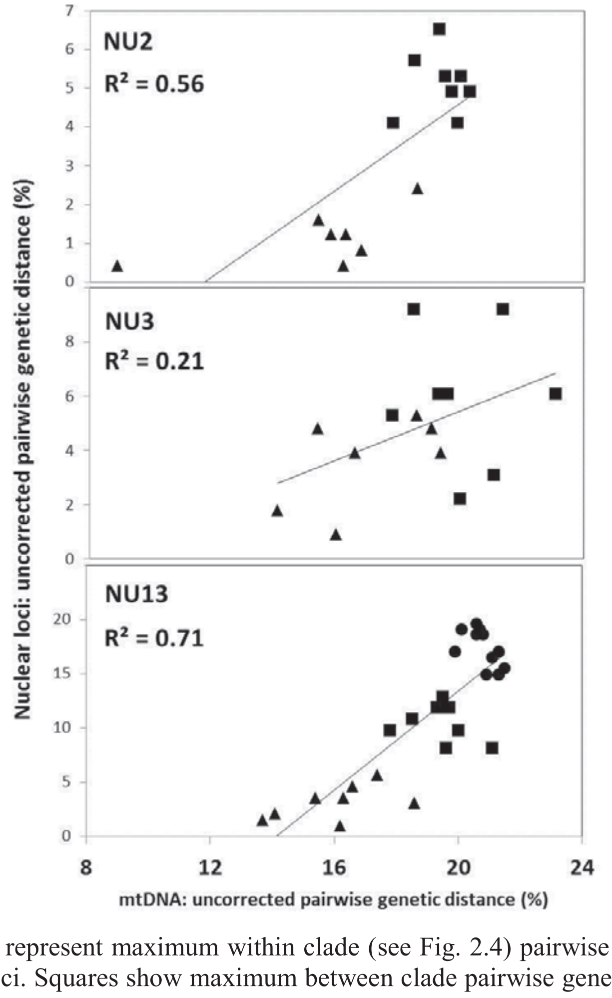

Figure 2.7. Triangles represent maximum within clade (see Fig. 2.4) pairwise genetic distances for each of the three nuclear loci. Squares show maximum between clade pairwise genetic distances. Circles show pairwise genetic distances between *Hemiandrus* and tree/giant wētā. For all three nuclear loci there was a significant correlation between mtDNA distances and nuclear distances. This relationship was weak for NU3 and showed an overlap between within clade distances and among clade distances whereas for NU2 and NU13 there was a stronger relationship and no overlap. Some overlap of intra- and interclade distances, however, is present within mtDNA.

Further support for vertical rather than horizontal transfer comes from the fact that alleles detected in different individuals of the same species were identical or separated by only one to three mutations (0.4-1.2%) (Fig. 2.8). The maximum difference between intraspecific alleles was within *H. maculifrons* and was concordant with mtDNA sequence analysis separating North Island and South Island populations. In most instances where more than a single individual of each species was included in the analysis, each species either had only a single allele or alleles formed a clade. In only one instance an allele was shared between species (NU2: *H. focalis* and *H. electra*) and on one occasion the alleles of one species were paraphyletic with respect to those of another species (NU13: *H. pallitarsis* and *H.* "timaru").

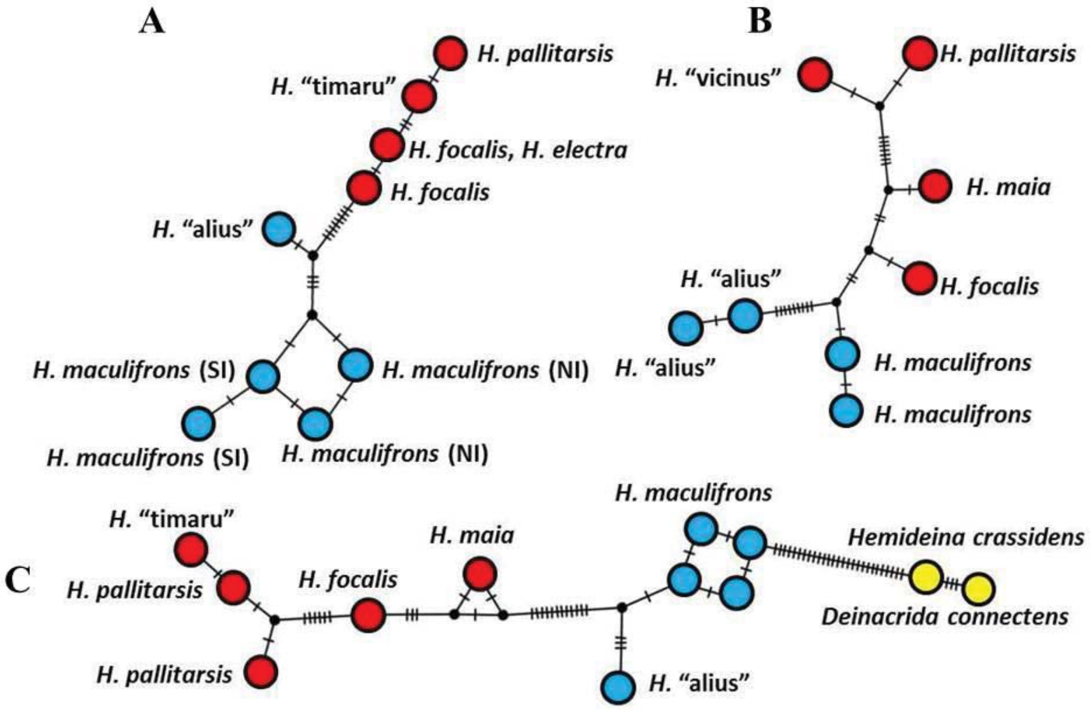

Figure 2.8. Median joining networks of nuclear alleles. **A**. NU2. **B**. NU3. C. **N**U13. Blue circles indicate alleles from individuals with mtDNA clade 1 haplotypes. Red circles indicate alleles from individuals with mtDNA clade 2 haplotypes. Yellow circles indicate alleles from outgroup species. Haplotypes from the same species are separated by few mutations, with a maximum of three mutations within a single species (*H. maculifrons*, NU2), but specimens from the same island were only separated by a single mutation. SI denotes South Island *H. maculifrons*, NI is North Island.

Discussion

I used next-generation sequencing data to design primers to amplify three nuclear DNA markers. The presence of fixed heterozygosity and reverse transcriptase domains indicates that all three loci are likely retrotransposons present in multiple copies in the genomes of these wētā. Retrotransposons are transposable elements (TEs); DNA sequences that are able to insert themselves into host genomes. TEs are a major component of eukaryotic genomes and variation in genome sizes between species is often due to differences in numbers of TEs (Le Rouzic *et al.* 2007). TEs enter the genome by horizontal gene transfer, hybridisation, or by recombination of existing elements. They then increase in number and spread in the genome. For example, Alu elements (retrotransposons) exist at more than a million copies in the human genome, although 99.5% of copies are monomorphic (Roy-Engel *et al.* 2001). The extent of spreading depends on where the transposon inserts in the genome, the nature of the TE and the host response (Le Rouzic *et al.* 2007). It also depends on the method of host reproduction (Abrusán and Krambeck 2006). Most TEs present in a host genome have been rendered inactive by mutations and deletions. In the *Drosophila melanogaster* genome, for example, less than 20% of transposable elements are full-length and even some of those that are full-length do not transpose but remain silent as cryptic elements (Slotkin and Martienssen 2007).

Retrotranspons have been used as phylogenetic markers in many instances (e.g. Cetartiodactyla, Nikaido *et al.* (1999); Strepsirrhini, Roos *et al.* (2004); Marsupiala, Nilsson *et al.* (2010)), but as binary markers with the presence of a specific retrotransposon in related species suggesting an orthologous relationship i.e. vertical transfer. Zerjal *et al.* (2012), however, looked at patterns of genetic variability in TEs (Miniature Inverted Repeat Transposable Elements (MITE)) in maize (*Zea mays ssp. mays*) and found that patterns of diversity and differentiation were in agreement with other makers (SNPs and SSRs) indicating that MITE polymorphisms reflect maize genetic history.

If the TEs studied in *Hemiandrus* were actively transposing and existing in many copies I would expect many alleles per individual and a lack of phylogenetic signal. I did not detect more than two distinct sequences within any single diploid wētā. All three nuclear markers showed congruence with mtDNA and with one another in both genetic distances and the topology of the phylogeny. These analyses suggest that the nuclear markers reflect *Hemiandrus* genetic history and are therefore likely vertically transferred. A mitochondrial phylogeny may not necessarily reflect the genetic history of a group of organisms due to processes such as incomplete lineage sorting and introgression. However, congruence with nuclear markers (morphological and TE sequences) is strong support for inferred evolutionary relationships.

Further support for vertical transfer of the TE markers is the fact that alleles found within a species were closely related to one another, and usually formed monophyletic clusters. In only a single case was an allele shared between species, an observation that would be much more common if these markers were actively transposing. The fact that most alleles were restricted to a particular species is evidence that these three markers are unlikely to be actively transposing elements, although in these situations, horizontal transfer cannot be ruled out. None of the wētā species sampled here are currently in contact with one another, and thus cannot directly transfer DNA, although past interspecific contact is possible.

Three markers were developed which fulfil the requirements of loci used for phylogenetic analysis: to exist as a single or low number of copies; to be orthologous; and to contain a suitable level of genetic variation (Alvarez and Wendel 2003). Two of the loci (NU2 and NU13) were present in outgroup species indicating that they originated in a common ancestor of *Hemiandrus*, *Hemideina* and *Deinacrida* rather than recently invading the genome (Fig. 2.6). A longer period since the invasion of a genome by a TE means more time for the TE to be rendered inactive by mutations. NU3 was not found in the outgroup species, indicating that either it entered the anostostomatid genome more recently, or that this region was not amplified during next-generation sequencing.

Conclusion

The three markers described here are likely retrotransposons. It is possibly they are present in multiple copies but only one or two sequence variants were detected in each individual. Tested over multiple species and clades, I found that the relationships of the nuclear markers reflect current taxonomy and mtDNA evidence, indicating that these three TEs are likely no longer transcriptionally active. For inferring low levels of gene flow, as expected of separate species, these markers are suitable as there is allelic variation, primers amplify orthologous elements, presence is due to common ancestry, and distinct alleles reveal independent lineages (that have not recently exchanged TEs). In conjunction with other markers/traits (in much the same way that the multi-copy ITS marker is used) these new nuclear markers could be used with caution for phylogenetic analyses and to elucidate species boundaries in ground wētā.

Appendix

Hemiandrus specimens used in this chapter. *Specimens used for next-generation sequencing.

Species	Code	Location
H. maculifrons	GWATENE (NGS)*	Atene (WI)
H. maculifrons	GW239	Taranaki (TK)
H. maculifrons	GW819	Kahuterawa (WN)
H. maculifrons	GW813	Kahuterawa (WN)
H. maculifrons	GW907	Pelorus (SD)
H. maculifrons	GW897A	Pelorus (SD)
H. maculifrons	GW897B	Pelorus (SD)
H. maculifrons	GW88A	Pelorus (SD)
H. maculifrons	GW550	Pureora (TO)
H. "alius"	GW896A	Denniston Plateau (NN)
H. "alius"	GW896B	Denniston Plateau (NN)
H. "alius"	GW896C	Denniston Plateau (NN)
H. "alius"	GW899	Denniston Plateau (NN)
H. "alius"	GW690	Flora carpark, Kahurangi National Park (NN)
H. focalis	GW8	Routeburn Track, Fiordland (OL)
H. focalis	FD6	Codfish Island (SI)
H. electra	GW96	Karamea River (NN)
H. electra	GW719	Awaroa (NN)
H. maia	GW764	Hampden, North Otago (DN)
H. pallitarsis	GWBLEDISLOE (NGS)*	Palmerston North (WN)
H. pallitarsis	GWBLEDISLOE (NGS)*	Palmerston North (WN)
H. pallitarsis	GWBLEDISLOE (NGS)*	Palmerston North (WN)
H. pallitarsis	GW176	Wellington (WN)
H. pallitarsis	GW320	Coromandel (CL)
H. "timaru"	GW211	Burkes Pass (MK)
H. "vicinus"	GW602	Te Rua Bay, Tory Channel (SD)
H. "vicinus"	GW54	Whites Bay, Blenheim (SD)

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Chapter 3. Marvellous *maculifrons*: discovering and interpreting genotypic clusters in a widespread ground wētā (Anostostomatidae: *Hemiandrus*).

Introduction

Species' ranges

Species' ranges are frequently dynamic, changing in size and position over time. It has been shown that range sizes of some taxa correlate with estimated time since their origin (Nattier *et al.* 2012), with species expanding their ranges early on before declining to extinction (Foote *et al.* 2007). Changes in range size and position may obscure patterns left by the speciation process but in at least some cases range size appears to remain stable after speciation and so may be informative (e.g. Ribera *et al.* (2011) and see chapter 1).

Although species' range size is subject to phylogenetic constraints (Abellán and Ribera 2011; Jablonski 2000), even closely related species can differ in the size of their range. The distribution of a species is influenced by many abiotic and biotic factors including geography, climate, morphological traits that affect mobility, life history traits that affect establishment, historical processes and species interactions. In addition, range size is likely to be affected by time and speciation events. Geographic location can affect range size because available niche space varies. Some locations are situated so that there is ample room for expansion, while other locations may be geometrically constrained by barriers such as oceans (Abellán and Ribera 2011). Furthermore, in terrestrial animal species, range size tends to increase with latitude (Rapoport's rule) (Stevens 1989), although this may be the result of many local phenomena rather than a single process (Gaston *et al.* 1998). Current range size may be a product of barriers that no longer exist, such as ice sheets during former glacial periods; some species may have yet to fill available habitat.

Species ranges are also influenced by interactions with other species. Darwin (1859) observed that “the struggle for existence” is stronger between more closely related species and concluded that this was because close relatives are more likely to occupy similar niches. On the other hand, species that are more phylogenetically and morphologically diverged are more likely to have reached the stage of secondary sympatry (i.e. secondary contact after speciation) (Pigot and Tobias 2013). Among New Zealand wētā, *Hemideina* tree wētā in North Island have ranges shaped by interspecific competition; the shifting of one species in response to environmental changes has been constrained by the range of a closely related species (Bulgarella *et al.* 2014).

Similar species may experience not only competition but also gene flow and even homogenization. When two closely related species are present in sympatry the integrity of each may be maintained through prezygotic barriers to gene flow such as morphological and behavioural differences or postzygotic barriers such as genetic incompatibilities due, for example, to coadapted alleles (Brideau *et al.* 2006; Butlin 1987; Dieckmann and Doebeli 1999). In many insects, male genitalia diverges rapidly due to sexual selection (Eberhard 1985), and such differences can be prezygotic barriers. The underlying mechanism of rapid genitalic evolution is usually unclear, although in most instances it is unlikely to have evolved primarily as an isolating mechanism (Hosken and Stockley 2004). Absence of gene flow is one of the defining features of the popular biological species concept (Mayr 1942), although hybridisation between species is not uncommon (Abbott *et al.* 2013).

New Zealand landscape and ground wētā

The New Zealand archipelago has been substantially reshaped by seismic activity during the last five million years, with the main islands acquiring their current shapes only in the last half million years (Trewick and Bland 2011). In chapter 1 I showed that historical seismic activity has impacted on the distribution of endemism and diversity. During global climate cycling in the Pleistocene, glaciation and lowered sea levels impacted on the New Zealand landscape and its biota. The last glacial maximum (LGM) between approximately 29 ka and 19 ka resulted in glaciers on mountainous parts of South Island (Alloway *et al.* 2007; Newnham 2007; Stevens 1980). The ranges of many species appear to have responded to cooler temperatures by tracking more hospitable climates and habitats. In South Island this resulted in species’ range disjunctions and recent range expansion between northern and southern South Island (Trewick and Wallis 2001). Also, some terrestrial taxa have ranges that span Cook Strait, a water barrier that is likely to limit gene flow between terrestrial organisms today. Connection of North and South Islands during the LGM by land exposed by lowered sea levels is the most probable explanation for such distributions.

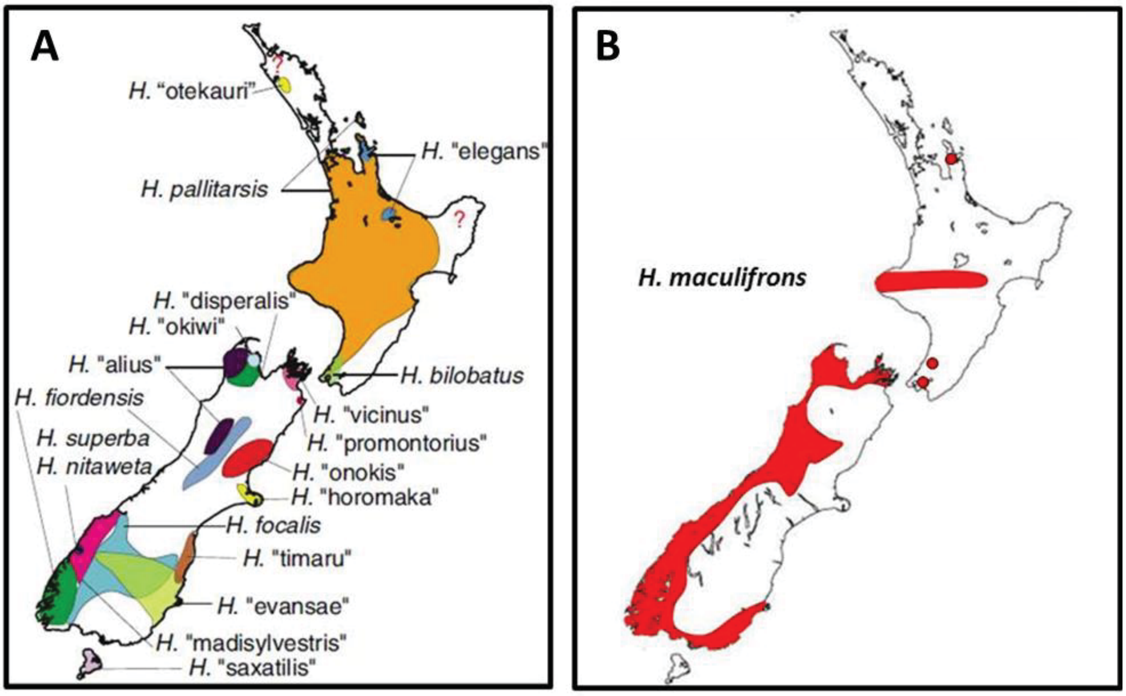


Figure 3.1. A. The distribution of New Zealand ground wētā. Species diversity in *Hemiandrus* is highest in South Island. Most species have narrow ranges. From Trewick and Bland (2011). **B.** The current understanding of the distribution of *H. maculifrons*, the only species with a range that extends across Cook Strait. Based on Johns (2001), Spurr & Berben (2004), Moeed & Meads (1985) and Chappell *et al.* (2014a, 2014b).

Ground wētā (*Hemiandrus*) are found throughout the main islands of New Zealand, as well as on numerous offshore islands (Fig. 3.1), but most species have restricted ranges. For example, *H. bilobatus* is found only in the Wellington region (Johns 2001) and *H. nitaweta* is restricted to part of Fiordland (Jewell 2007). In contrast, *H. maculifrons* (Walker 1869) has a range that extends across North and South Islands. *Hemiandrus maculifrons* is not only the most widespread ground wētā species, it is also the most widespread New Zealand anostostomatid. Preliminary data on *H. maculifrons* indicate high levels of genetic diversity (Pratt *et al.* 2008), but deep phylogeographic breaks within species are not uncommon and can arise stochastically (Bond and Stockman 2008). In fact, high genetic distances have been reported in other New Zealand anostostomatids, for which other evidence refutes the existence of multispecies. For example, the widespread North Island ground wētā, *Hemiandrus pallitarsis*, has been shown by genetic, morphological and acoustic analyses to be a single species even though mtDNA diversity is high (Chappell *et al.* 2012). Likewise, the North Island tree wētā *Hemideina thoracica* and the alpine scree wētā *Deinacrida connectens* have high genetic diversity but each is recognised as a single species (Bulgarella *et al.* 2014; Morgan-Richards 1997; Trewick *et al.* 2000; Trewick 2001; Trewick *et al.* 2011).

Hemiandrus maculifrons is found in primary native forest, and it is known that such habitat was less continuous in the recent past (~22,000 years) due to global climate cooling (Alloway *et al.* 2007). Some New Zealand forest insects show a pattern of recent range expansion including *Argosarchus horridus* (Buckley *et al.* 2009), *Clitarchus hookeri* (Buckley *et al.* 2010; Morgan-Richards *et al.* 2010) and *Hemideina* spp (Bulgarella *et al.* 2014). However, the retention of high genetic variation in other taxa suggests they survived the LGM and probably earlier glacial cycles in numerous, widespread populations (e.g. *Asplenium hookerianum*, Shepherd *et al.* 2007). Thus it is possible that *H. maculifrons* retained a wide distribution in New Zealand throughout climate cycling or gained a wide range through recent but extensive range expansion from few refugia.

Here I use the distribution and extent of genetic diversity within *H. maculifrons* to test whether the unusually wide-ranged *H. maculifrons* is consistent with it being a single species or whether it comprises several cryptic taxa. Species have arisen under different evolutionary scenarios and so have different species-associated properties (de Queiroz 2007). Here I define "species" using the genotypic cluster approach (Mallet 1995), according to which species are groups of individuals identified by morphology or genetics that have few or no intermediates when in contact. The benefit of the genotypic cluster definition is that it is an operational definition providing a testable hypothesis of species boundaries without relying on interbreeding or the lack of it. When populations are not in contact with each other then concordance of characters that produce "clusters" of individuals can provide a means to assign "species" status. Defining species as separately evolving metapopulation lineages (de Queiroz 1998) means that any divergent character can be considered as evidence of a potential species boundary and therefore does not provide a reasonable testable species hypothesis. If *H. maculifrons* consists of more than a single taxon it is likely to comprise concordant genetic and morphological differences between clades that are maintained in sympatry. The sharing of some alleles might be due to incomplete lineage sorting (ILS) and low levels of gene flow, and could still be compatible with distinct genotypic clusters.

To assess the nature of diversity within *H. maculifrons* I identify mtDNA clades and examine their significance using nuclear markers, morphological data and clustering tools to test the null hypothesis that *H. maculifrons* is a single morphologically homogeneous unit.

Methods

Hemiandrus maculifrons (Walker 1869)

This small forest ground wētā species is sympatric with many congeners (pers obs). It has been reported throughout much of South Island (10 of 15 Crosby regions (Crosby *et al.* 1976)), and on North Island in Taranaki, the Kaweka Ranges, Tongariro National Park (Johns 2001), the western Tararua Ranges (Spurr and Berben 2004), the Orongorongo Valley (Moeed and Meads 1985) and on the Coromandel Peninsula (Chappell *et al.* 2014a; Chappell *et al.* 2014b). It feeds mainly on small invertebrates and lives in moss, soil, leaf litter and under logs with measured densities at 0.7-1.6 per m² (Cary 1981). Unlike other species that have maternal care of eggs and

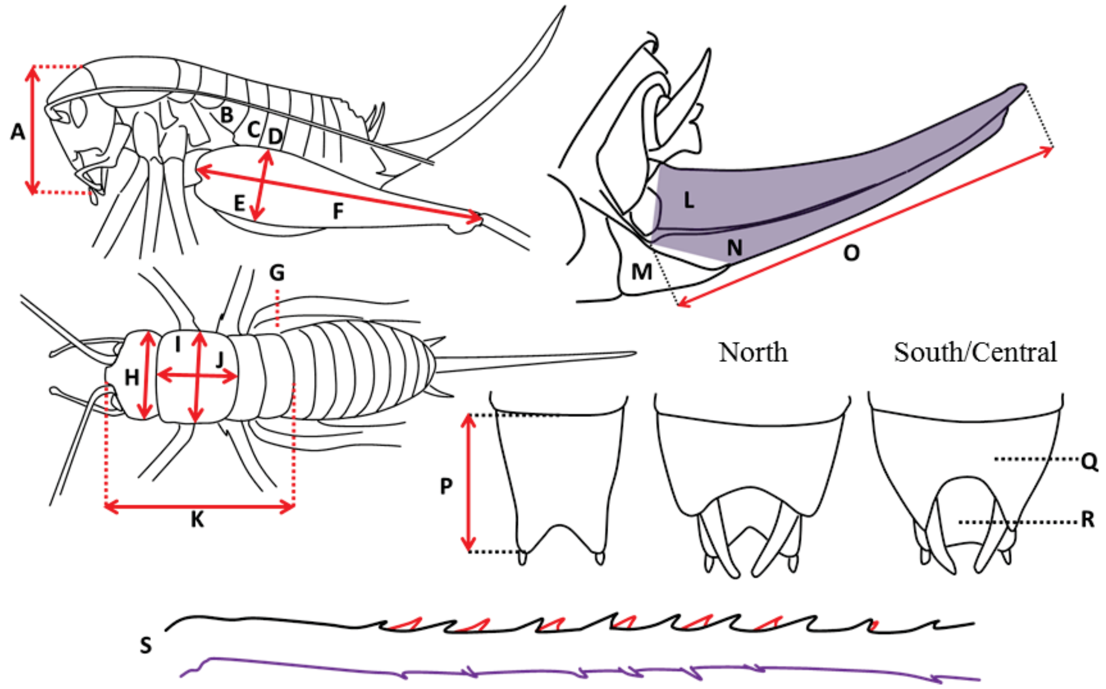


Figure 3.2. Eight measurements and counts of pegs/spines in seven different positions on the body were made of each specimen. For females, ovipositor shape and for males the shapes of the subgenital plate and ninth abdominal tergite (T9) were examined. The lateral view of a female ground wētā shows: A. Head length (HL). B. Position of first abdominal tergite (T1) stridulatory pegs. C. Position of T2 pegs. D. Position of T3 pegs. E. Femur width (FW). F. Femur length (FL). The dorsal view of a female ground wētā shows: G. Position of stridulatory pegs of the hind femur. H. Head width (HW). I. Pronotum width (PW). J. Pronotum length (PL). K. A proxy for body length (BL): distance from frons to distal margin of metanotum. The lateral view of the female ovipositor shows the shape analysed in the elliptic Fourier analysis (purple) as well as: L. The third valvula of the ovipositor. M. Female subgenital plate. N. First valvula of the ovipositor. O. Ovipositor length (OV). Dorsal and ventral views of male terminalia show: P. Male subgenital (SG) plate length (ventral angle). Q. Ninth abdominal tergite (T9) shape (North lineage males had rounded lobes while South and Central lineage males had sharp lobes). R. Subgenital plate- dorsal angle (North lineage males had a U or V shaped distal edge while other males had a flat or slightly concave edge). S. Lateral view of the hind femur showing its spines. Black: superior prolateral fixed spines. Red: superior retrolateral fixed spines. Purple: inferior articulated spines.

nymphs or abandon their eggs in soil above ground, *H. maculifrons* has a long ovipositor (Fig. 3.2) which it uses to insert its eggs into the walls of their galleries (Cary 1981). Chappell *et al.* (2014b) found that males are smaller than females and also more frequently encountered. Individual *H. maculifrons* moved up to 70m per night (Chappell *et al.* 2014b).

Collection of specimens

Hemiandrus maculifrons specimens were captured by night searching and pitfall trapping in various habitats throughout both North and South Islands. All specimens collected were lodged in the Phoenix Lab insect collection at Massey University. Specimens from Eric Edwards (Department of Conservation) were also examined. All Phoenix Lab *H. maculifrons* were collected between 1997 and 2014 and preserved in 95% ethanol. Specimens of *H. maculifrons* were differentiated from other *Hemiandrus* species based on body colour and patterning, pilosity of tarsi, pilosity of maxillary palps, male terminalia and body size (Jewell 2007; Johns 2001). Males were assessed to be adults based on the presence of dark, sclerotized hooks (falci) beneath the ninth abdominal tergite (T9) while females were assessed to be adults based on the shape of the subgenital plate and the sclerotization of the ovipositor (Cary 1981).

DNA extraction, amplification & sequencing

Muscle tissue from a leg of each specimen was dissected out and total genomic DNA extracted using a salting-out extraction method (Sunnucks and Hales 1996). A fragment of the mitochondrial cytochrome oxidase I gene (COI) was amplified using primers Mtd10crass and 12wetaR (from Bulgarella *et al.* 2014). Three nuclear loci were amplified using primers designed in chapter 2. Polymerase chain reaction (PCR) used the following conditions: 94°C for 2 min then 35 cycles of 94°C for 30s, annealing at 55-59°C for 30s, and extending at 72°C for 30-60s; 72°C for 8 min. Amplified DNA products were treated to Shrimp Alkaline Phosphotase⁄Exonuclease digestion (USB Corp.) prior to sequencing to remove unincorporated primers. Cycle sequencing with the PCR primers used Bigdye chemistry (PE) following the manufacturer's protocols, with automated analysis on an ABI3730. Sequences were edited and aligned using Geneious v6.1.6 (Kearse *et al.* 2012).

Phylogenetic analysis

Phylogenetic analysis of the COI alignment used published sequence from *Hemiandrus pallitarsis* as the outgroup. 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 jModelTest v2.1.3 (Darriba *et al.* 2012). Bayesian phylogenetic analysis was performed using a transversion model of molecular evolution incorporating invariant sites and a gamma distribution (TVM+I+G model) in BEAST v1.8 (Drummond *et al.* 2012). Two BEAST analyses were run for 50 million generations each using a coalescent tree prior, with a burn-in of 5 million generations for each run. Output statistics were examined using TRACER v1.5.0 (Rambaut and Drummond 2009). The runs were combined using LogCombiner v1.8 (Drummond *et al.* 2012) and trees were visualised using FigTree v1.4 (Rambaut 2012). Phylogenetic analysis was also performed using the maximum-likelihood (ML) approach (Felsenstein 1981) in RAxML (Stamatakis 2006) for 500 non-parametric bootstrap iterations using the GTR + I + G model.

Nuclear-mitochondrial pseudogenes (Numts) are common in some insects and are reported in some ground wētā (Chappell *et al.* 2012). Chromatogram traces for COI mtDNA that had double peaks suggesting more than one sequence had been amplified (Fig. 3.3) were excluded from the data set as this can be the result of the co-amplification of mitochondrial and nuclear copies.

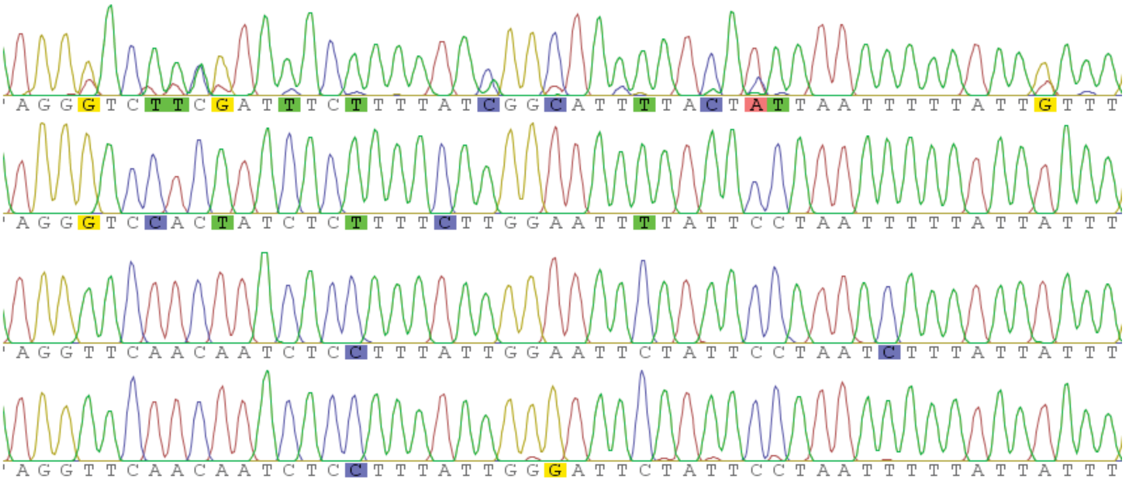


Figure 3.3. Nuclear-mitochondrial pseudogenes (numts) are common in insects and are present in ground wētā (Chappell *et al.* 2012). This section of COI mtDNA chromatogram shows a probable numt (nuclear mitochondrial pseudogene; top trace) i.e. nuclear copies amplified and sequenced along with mtDNA resulting in double peaks. The three traces below have no double peaks and represent mtDNA only.

Wētā specimens with similar mtDNA haplotypes were grouped (= clades) and were tested for properties associated with species boundaries only if they formed well-supported clades in both the Bayesian and the ML trees. Phylogenetic relationships were considered strongly supported in Bayesian analysis when posterior probabilities were ≥0.95 and in the ML analysis when bootstrap proportions were ≥70% (Alfaro *et al.* 2003; Hillis and Bull 1993). Using the "species delimitation" tool implemented in Geneious (Masters *et al.* 2011), statistics were generated from the ML tree to assess the strength of phylogenetic parameters associated with putative species boundaries. These software tools are resampling techniques that assess confidence in the inferred monophyly and clustering of DNA sequences (Appendix 3.1). Mean within and between clade genetic K2P-corrected genetic distances (Kimura 1980) were also calculated in MEGA v6.0 (Tamura *et al.* 2013).

Haplotype networks for three main lineages identified by phylogenetic analysis were constructed using a shorter alignment of COI sequences (501bp), representing more *Hemiandrus maculifrons* individuals, from more locations. Networks were constructed in PopArt (popart.otago.ac.nz) using statistical parsimony analysis (TCS) (Templeton *et al.* 1992).

Alleles of three nuclear loci were reconstructed using the PHASE algorithm (Stephens and Donnelly 2003; Stephens *et al.* 2001) implemented in DnaSP v5 (Librado and Rozas 2009). PHASE uses a coalescent based Bayesian method to infer alleles. Allele networks were constructed using statistical parsimony analyses in PopArt. A multilocus, coalescent species delimitation analysis was conducted using the three nuclear loci and mtDNA in the program BPP v2.2 (Rannala and Yang 2003, 2013; Yang and Rannala 2010) (Appendix 3.1). This analysis was used on clades with at least two sequences for each of the four loci. This analysis was repeated for two datasets: the first with IUPAC ambiguity codes representing heterozygous sites in nuclear DNA and the second with the phased nuclear alleles for each locus treated as separate loci.

Morphology

Count and measurement data

Hemiandrus maculifrons specimens were examined using an Olympus SZX7 Zoom Stereomicroscope with an SC100 digital camera, and measured using digital callipers and Olympus Image Analysis Software. Eight measurements were made of each (Fig. 3.2): body length (BL: distance from frons to distal margin of metanotum), head width (HW), pronotum width (PW), femur length (FL), femur length (FW), pronotum length (PL), head length (HL), female ovipositor length (OV) and male subgenital plate length (SG). I also recorded the number of inferior articulated spines and superior fixed spines on prolateral and retrolateral angles of the mid tibiae as an average of both legs; the number of stridulatory pegs on the right hind femur; the number of pegs on the first three abdominal tergites on the right side of the body; the shape of the male T9; the shape of the male SG; the size of the lateral patches on the pronotum and the presence of dorsal pronotum patterning.

Statistical analyses

Standard descriptive statistics and analyses of morphometric data were undertaken using R v3.1.3 (R Development Core Team 2010). Because variation at several traits did not follow a normal distribution (Shapiro-Wilk normality test; <0.05), measurement data were log transformed and the count data square root transformed. Count and measurement data were analysed separately.

Paired *t*-tests were used to confirm that males and females differed in body measurements. The variability of morphological characters between clades was analysed using univariate and multivariate methods. Univariate comparisons were performed by one-way analysis of variance (ANOVA). If significant differences ($p<0.05$) were found among clades, then the Tukey-Kramer post-hoc test was applied to determine where the differences lay. Multivariate analyses included principal component analysis (PCA) that summarises multivariate information and allows the detection of similarities and differences in the data. Analysis included eight body measures and also, for females, the length of the ovipositor scaled to femur width, and for males, the length of the subgenital plate scaled to pronotum length. A linear discriminant function analysis (DFA) with cross validation was performed to determine whether the measurement data could accurately predict clade membership. Clades represented by a single specimen were included in the PCA but were excluded from the ANOVA and DFA.

I carried out a “Gaps in Continuous Characters across Geography” (GCCG) analysis (Zapata and Jimenez 2012) (Appendix 3.1) to assess the strength of evidence of a gap between morphological characters rather than simply a difference in central tendency. This method was applied to PC1 and PC2 of males and females separately.

Paired *t*-tests were used to reveal which discrete characters differed significantly between sexes. Those that were not significantly different between sexes ($p>0.05$) were pooled and analysed together whereas those that were significantly different were analysed independently. Univariate comparisons between clades were performed by Kruskal-Wallis one-way ANOVA. If significant differences among the populations were found ($p < 0.05$), then Dunn’s post-hoc test was used to determine where the differences lay.

Geometric morphometric analyses

Variation in ovipositor shape was assessed using geometric morphometric (GMM) analysis. The lateral view of the ovipositor was photographed for 76 females. Damaged ovipositors were excluded from the dataset. The shape analysed terminated ventrally at the anterior of the ovipositor where the subgenital plate meets and first valvula, and dorsally on the 3rd valvula perpendicular to the margin of the first valvula (Fig. 3.2). The software package SHAPE v1.3 (Iwata and Ukai 2002) was used to perform an elliptic Fourier analysis (EFA) (McLellan and Endler 1998) to reduce two-dimensional outlines into elliptic Fourier descriptors (EFD) that could be analysed statistically (Kuhl and Giardina 1982). The contour shape was described in the first 20 harmonics of Fourier coefficients. Principal component analysis was performed on the resulting normalised EFD coefficients.

The relative position of the mid tibial spines has been used for species identification of ground wētā (Johns 2001). Although the position of the spines of *H. maculifrons* has been described as "almost paired" (i.e. symmetrically positioned), there appeared to be high variation in spine position (pers obs). I examined the relative positioning of spines using GMM analysis. Initially, left and right mid tibiae of 31 individuals were each photographed twice. Legs that were damaged or missing were excluded from the analysis. Using TpsDig2 (Rohlf 2006), seven landmarks were placed on each image centrally above each pro and retro-dorso lateral spine. MorphoJ (Klingenberg 2011) was used to implement Procrustes ANOVA comparing separate images of the same leg and the two legs of the same individual. This showed no significant imaging error (F=0.23, p=1) and no difference between left and right legs (F=0.28, p=0.99). An additional 36 individuals were measured with a single image of the right leg only (unless missing or deformed). As there was also no significant difference between sexes (F=0.27, p=0.98) males and females were analysed together. Regression analysis was used to remove allometric effects. A covariance matrix was generated using regression residuals and a PCA performed. "Gaps in Continuous Characters across Geography" (GCCG) was also applied to the first two principal components of ovipositor shape and mesotibial spine position separately.

Results

Collection and identification of specimens

Hemiandrus maculifrons specimens were collected and identified from many more locations than previously recorded, expanding the known range to locations throughout North Island and northern South Island (Appendix 3.2). All specimens were collected from primary endemic forest.

mtDNA

The COI mtDNA alignment of 740 base pairs represented 132 *Hemiandrus maculifrons* specimens and comprised 304 (41.1%) variable, and 247 (33.4%) parsimony informative positions. Translation of the sequence confirmed it was derived from the coding DNA of the mitochondria. Amino acid changes were observed at 60 of the 246 codons. Ambiguous chromatogram traces suggesting the possibility of co-amplification of mitochondrial and nuclear copies (numts) of COI were present for three wētā from three different populations and these were excluded from the analysis. To increase the number of wētā specimens included, an alignment of 501bp of COI mtDNA with an additional 26 specimens was used, giving a total of 159 individuals for haplotype network analysis.

The mtDNA Bayesian phylogeny indicated that the sampled *H. maculifrons* consisted of three main lineages (North lineage, South lineage and Central lineage) (Fig. 3.4). The maximum genetic distances between these three lineages was 0.34 (GTR+I+G), 0.23 (K2P) and 0.17 (uncorrected). I observed three amino acid changes shared by North lineage specimens separating them from Central and South lineages. Two amino acid changes were shared by individuals of Central lineage separating them from South lineage. Nested within these three lineages were nine clades that each had high statistical support and that were also supported by ML analysis. Thus wētā specimens were grouped into one of nine clades. One “clade” (North D) was represented in the phylogenetic tree by a single individual but was considered as a separate group as it was sister to a well-supported, clade. South B was represented by two individuals that had the same haplotype but was considered a separate group due to the high support of its sister groups in both Bayesian and ML analyses. North lineage (n=64) consisted of three clades from wētā sampled throughout North Island and one clade (North C) collected from northern South Island. Individuals collected from the same location usually had mtDNA haplotypes from the same clade but there were a few exceptions. Members of two clades (North A and North D) were sympatric at Pureora Forest Park in the middle of North Island (Fig. 3.5). Central lineage (n=29) consisted of a single well-supported clade comprising haplotypes from wētā collected in southern North Island and northern South Island. Bayesian analysis supported two clades within Central, although ML did not. Representatives of the Central lineage were sympatric with wētā from four different clades at three locations. South lineage (n=66) consisted of four clades, representing wētā from South Island. Two clades (South A and South C) were sympatric with one another and with wētā with mtDNA haplotypes from the Central clade at Lewis Pass (BR).

One individual *Hemiandrus* wētā collected at Paturau River (NN) looked similar to other *H. maculifrons* but was genetically distinct from all others (Fig. 3.4). Due to the absence of other samples from this region it was excluded from subsequent analysis. The ML tree (not shown) had a similar overall topology as the Bayesian tree, with the exception that the inferred relationships of the singleton from Paturau River differed: the ML tree suggested the Paturau River wētā was sister to South lineage rather than sister to all other *H. maculifrons* as inferred by the Bayesian analysis.

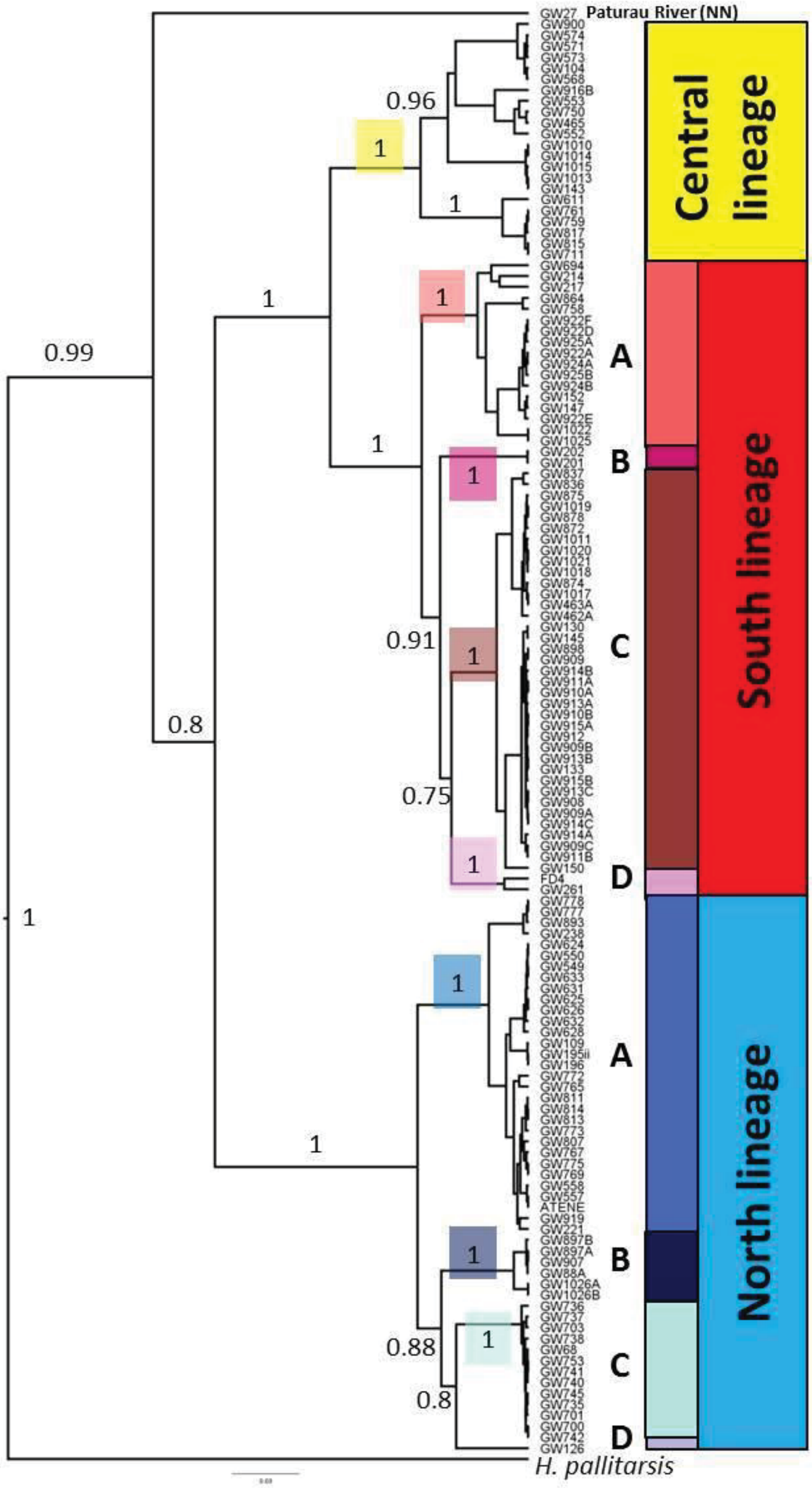


Figure 3.4. Bayesian mtDNA COI phylogeny of 132 *H. maculifrons* specimens and with *H. pallitarsis* as the outgroup. Three main lineages (North lineage, South lineage and Central lineage) are apparent. Nested within these three lineages are nine strongly supported clades with Bayesian analysis posterior probabilities ≥0.95 (coloured squares), ML analysis bootstrap proportions ≥70% (not shown). One individual collected at Paturau River (NN) looked similar to other *H. maculifrons* but was genetically distinct from all others.

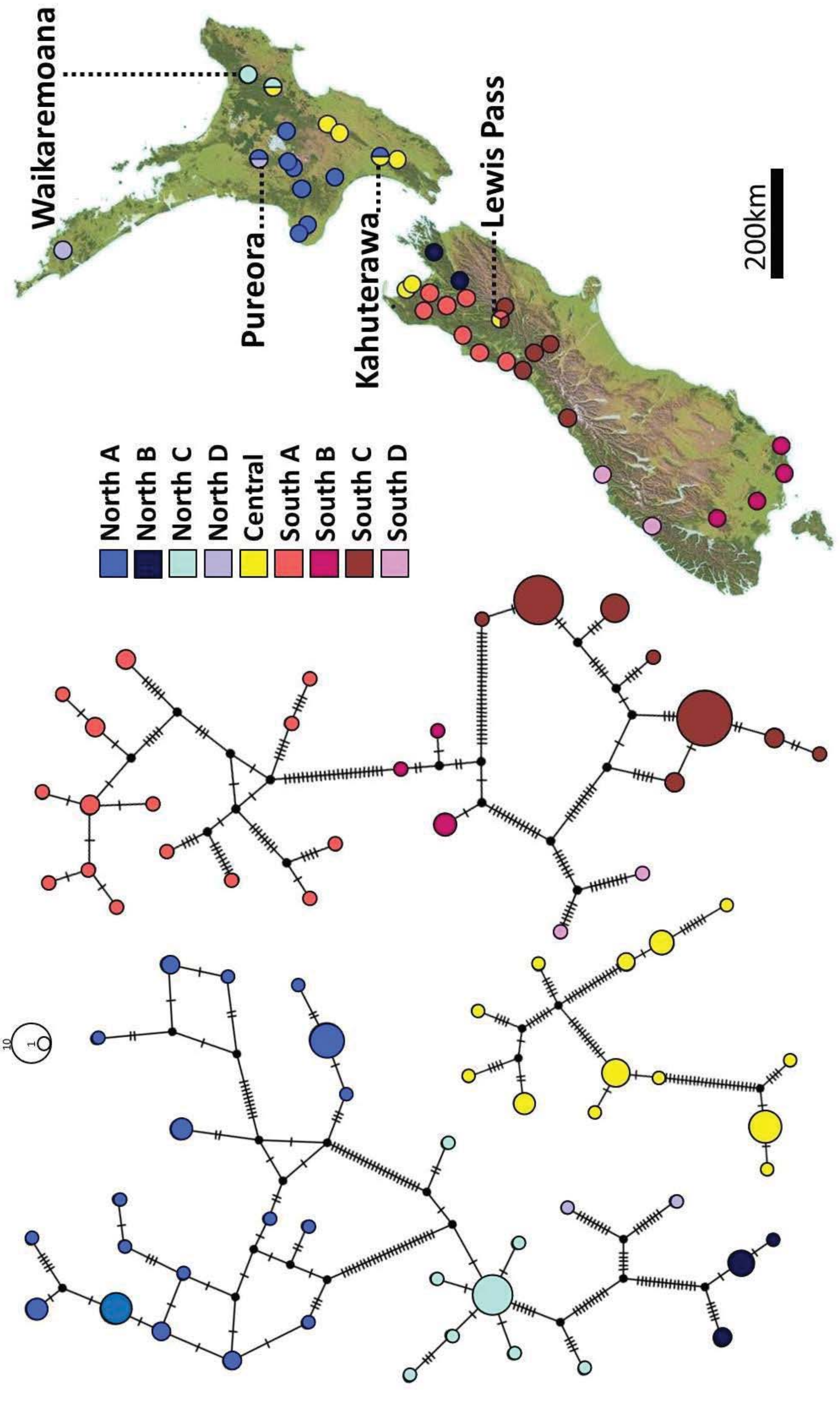


Figure 3.5. Haplotype networks from alignment of 501bp mtDNA COI sequence for 159 individuals. North lineage (blue) comprised four clades in North Island and northern South Island. Central lineage (yellow) comprised a single consistently clade in the axial ranges of North Island and northern South Island. South lineage (red) comprised 4 clades in South Island. Sympatry between clades was detected at four locations: Waikaremoana (North C and Central), Pureora (North A and North D), Kahuterawa (North A and Central) and Lewis Pass (Central, South A and South C).

"Species delimitation" analyses

Species delimitation statistics assess the strength of phylogenetic parameters associated with putative species boundaries and whether the apparent exclusivity of mtDNA clades could be attributed to the stochasticity associated with the coalescent process. I found that pairwise genetic distances calculated based on the phylogeny varied greatly depending on the specimens included, the model used and the method of tree construction. Therefore all genetic distances reported are K2P distances calculated in MEGA v6.0. The Geneious species delimitation statistics were unaffected by this variation and so were calculated based on the maximum likelihood phylogeny.

Most statistics applied here supported the nine mtDNA clades as distinct clusters (Table 3.1). This is not surprising as clades were identified on the basis that they were reciprocally monophyletic in the phylogenetic analyses. Rosenberg's (2007) P(AB), which gives the probability of reciprocal monophyly of two clades under a model of random coalescence, indicated that the monophyly of all the clades was not stochastic ($p \leq 0.01$) and therefore unlikely to be the result of random branching of lineages within a single species. On the other hand, the phenetic test of genetic distinctiveness, P(Randomly distinct) (Rodrigo *et al.* 2008), indicated that the high genetic structure on long branches could be the result of stochastic coalescence ($p \geq 0.05$). P(Randomly distinct) is based on a coalescent model where the species is panmictic and of constant size, while P(AB) is based on a null model of random coalescence and so these methods may not be appropriate for taxa that have histories of population isolation and range restriction.

Mean K2P corrected within-clade genetic distance ranged from zero (South B) to 0.06 (Central and North lineages). The highest mean among lineage K2P corrected genetic distance was between North and Central lineages (0.18). The ratio of within clade to among clade (i.e. to closest clade) genetic distance ranged from 0.03 (North C) to 0.46 (Central). A lower ratio indicates more differentiation between clades, but this statistic is highly dependent on sampling and has no clear threshold for delimitation.

Table 3.1. Sample sizes, phylogenetic branch support, species delimitation statistics (see Appendix 3.1), number of specimens available for morphological analysis and nuclear loci obtained and analysed for *H. maculifrons* in nine clades and three main lineages. Genetic distances are measured in substitutions per site. *Test not valid for sample sizes of more than 40 individuals. ** single or identical sequences.

Clade/ Lineage	mtDNA sample size		Branch support		"Species delimitation" statistics							Morphology		Nuclear loci	
	740 bp	501 bp	Bayesian	ML	Closest clade	Mean K2P intra distance	Mean K2P inter distance	Intra/ Inter	P ID(Liberal)	P (Randomly Distinct)	Rosenberg's P(AB)	Females	Males	All three nu loci	Bayesian species delimitation supported?
North A	31	37	1	100	North C	0.02	0.09	0.22	0.97 (0.95, 1.0)	1	≤0.001	16	13	Y	Y
North B	6	7	1	99	North D	0.01	0.06	0.11	0.93 (0.79, 1.0)	0.05	≤0.001	0	0	Y	Y
North C	13	17	1	97	North D	<0.01	0.06	0.03	0.97 (0.87, 1.0)	0.05	0.01	10	5	None	NA
North D	1	3	NA**	NA**	North B	NA	0.06	NA	0.96 (0.83, 1.0)	NA	0.01	3	0	Nu2, Nu3	NA
South A	17	18	1	95	South B	0.03	0.07	0.39	0.96 (0.91, 1.00)	0.72	≤0.001	8	1	Y	Y
South B	2	5	NA**	NA**	South D	NA	0.06	NA	0.96 (0.83, 1.0)	NA	≤0.001	4	0	None	NA
South C	37	41	1	96	South D	0.02	0.07	0.22	0.97 (0.94, 1.00)	0.05	≤0.001	23	5	Y	Y
South D	2	2	1	99	South B	0.02	0.06	0.40	0.77 (0.61, 0.92)	1	≤0.001	1	0	Y	NA
Central	22	29	1	92	South	0.06	0.13	0.46	0.95 (0.91, 0.99)	0.8	≤0.001	17	5	Y	Y
North lineage	51	64	1	95	Central	0.06	0.18	0.31	0.97 (0.94, 1.00)	NA*	≤0.001	29	18	Y	Y
South lineage	58	66	1	79	Central	0.05	0.13	0.39	0.96 (0.94, 0.99)	NA*	≤0.001	36	6	Y	Y

P ID(Liberal) (Ross *et al.* 2008) gives the mean probability (95% confidence interval) of a member of a specified clade fitting inside or at least sister to the clade comprising the other individuals belonging to this species. These statistics depend on the intra-inter genetic distance ratio and the number of individuals sampled so that abundant sampling of individuals and a low ratio results in a good chance of correct ID. Kiewnick *et al.* (2014) applied a threshold P ID(Liberal) value of ≥0.93 for a species to be considered “adequately delimited”. This was based on Hamilton *et al.* (2014) who observed that all but one (of 54) delimited species had a P ID(Liberal) value of ≥0.93 (the other delimited species was 0.81). Other authors have applied a value of ≥0.90 (e.g. López-López *et al.* 2012). The value selected will depend on the system being investigated and therefore should only be used as a guide. Here, all clades had values higher than 0.93 except for South D (0.77), which had a small sample size and a high within clade to among clade ratio.

Bayesian species delimitation indicated that for all prior parameter combinations the model specifying all five *H. maculifrons* clades analysed (Table 3.1) as five species had posterior speciation probabilities of one.

Nuclear loci

Alignments of DNA sequences were obtained for three anonymous nuclear loci (chapter 2). The NU2 locus alignment of 45 individuals and 247 bp contained 17 (6.9%) variable sites all of which were parsimony informative. This locus had more genetic diversity than the other two nuclear loci. The NU3 locus sequence alignment of 50 individuals and 228 bp contained 8 (3.5%) variable sites all of which were parsimony informative, and the NU13 locus alignment of 50 individuals and 194 bp contained 10 (5.2%) variable sites all of which were parsimony informative. Genotypes for all three nuclear loci were obtained for wētā representing seven of the nine mtDNA clades (Table 3.1).

Wētā from each of the three main mtDNA lineages had private alleles at each of the three nuclear loci, but there was sharing of alleles between clades (Fig. 3.6). For the North lineage wētā all but one mtDNA clade (North C) were represented. No nuclear alleles were shared between wētā in the North lineage and any other main lineage. Although there was evidence of sharing between clades North A, North B and North D, each of these three clades had at least one private allele (Fig 3.6). Wētā with Central and South mtDNA lineages shared some nuclear alleles, although Central lineage wētā also had some private alleles for all three nuclear loci. For the South lineage wētā all but one mtDNA clade (South B) was represented. South A and South C also had private alleles but all South D alleles were shared. Therefore the distribution of nuclear alleles was consistent with the three main groupings indicated by mtDNA. Sharing of some nuclear alleles by Central and South mtDNA lineage wētā is consistent with their sister relationship in Bayes and ML phylogenies (Fig 3.6).

At Lewis Pass, where mtDNA clades Central, South A and South C were in sympatry, South A and South C shared alleles at all three nuclear loci (n = 2 and 8), but wētā with Central haplotypes had fixed differences at two loci (NU2, NU3; Table 3.2).

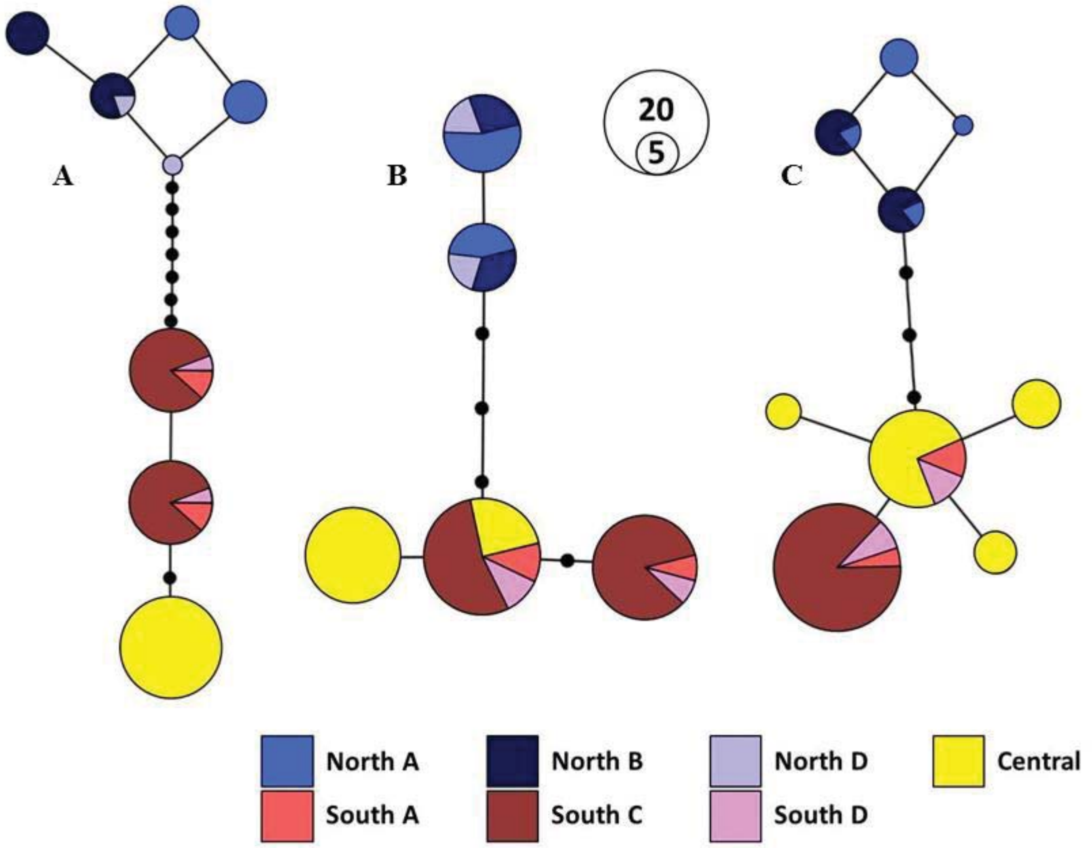


Figure 3.6. Networks representing allele diversity at three anonymous nuclear loci in *Hemiandrus*. Nuclear DNA allele networks. **A**. NU2. **B.** NU3. **C.** NU13. Colours represent major mtDNA lineages. Wētā from each of the three mtDNA lineages had some private alleles at each of the three nuclear loci and all North clades analysed (A, B and D) had some private alleles. South and Central lineages shared some NU3 and NU13 alleles, but they shared no NU2 alleles- the nuclear locus which had the largest proportion of parsimony informative sites. At Lewis Pass, where mtDNA clades Central, South A and South C were in sympatry, South A and South C shared alleles for NU3 and NU13 but Central had only private alleles (Table 3.2). For nuclear locus NU13, South A shared alleles with both South C and Central, and although South C had no private alleles, Central did.

Table 3.2. Lack of gene flow between *Hemiandrus maculifrons* mtDNA Central and South clades where they are sympatric at Lewis Pass, South Island as revealed by nuclear markers.

mtDNA clade	NU2		NU3		NU13	
	Sample size	Alleles	Sample size	Alleles	Sample size	Alleles
Central	4	A	5	A	6	A, B
South A	1	B, C	2	B, C	2	B, C
South C	7	B, C	7	B, C	8	C

Morphology

Qualitative characters

I examined the morphology of 111 adult putative *H. maculifrons* ground wētā for which I had genetic data. This included 82 adult females and 29 adult males (Table 3.1). Analysis of male terminalia shape showed two distinct types of terminalia within the *H. maculifrons* sample (Fig. 3.2, Fig. 3.7). North lineage males had a sinuous distal T9 margin whereas other males had lobes with sharp margins (Table 3.3). Male wētā representing the different mtDNA lineages also differed in the shape of their subgenital plates (SG); the margin of North lineage subgenital plates was concave ranging from a shallow curve to a deep "V", while other males had subgenital plates with flat or slightly curved margins. The V and U shapes probably represent variation on a continuum as both shapes were found within a single population with North C haplotypes.

The patterning of the pronotum was found to be distinct between some lineages (Fig. 3.7). North lineage wētā had pronota with or without pale dorsal patterning and small or no lateral patches on each side, while South and Central lineage wētā had mottled pronota with large pale lateral patches.

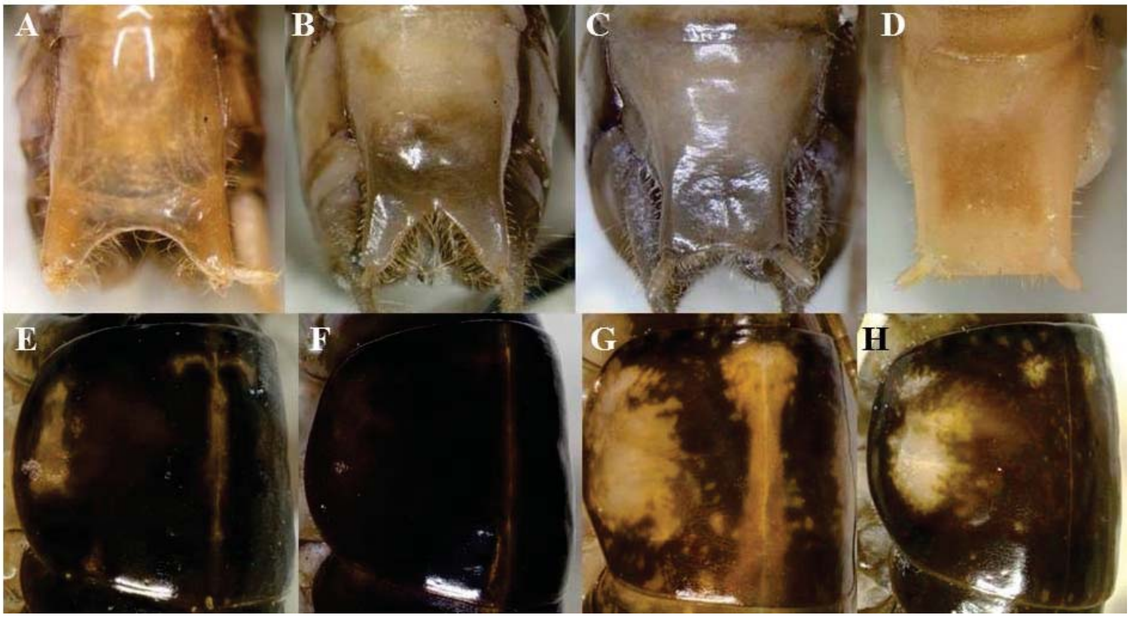


Figure 3.7. Variation in *Hemiandrus maculifrons* morphology. Two distinct types of male terminalia were identified (**A-D**). The subgenital plates of North lineage males had a concave margin which ranged from a shallow curve (**A**) to a deep “U” or “V” (**B**), while other males had subgenital plates with slightly curved (**C**) or flat margins (**D**). Pronotum colouring also varied among members of mtDNA lineages (**E-H**). Northern individuals had small (**E**) or no (**F**) lateral patches close to the edge of either side, while South and Central lineage wētā had large pale lateral patches close to the edge of either side (**G**, **H**).

Quantitative characters

Many morphometric variables differed significantly among males of five clades and among females of seven clades using one-way ANOVA (Table 3.3). A Tukey-Kramer post-hoc test was used to determine where the differences lay. These tests were also applied to specimens grouped into the three main lineages.

Males of five mtDNA clades analysed differed significantly from one another in all body measurements ($p \leq 0.005$) (Table 3.4). The most significant difference was in subgenital plate length, both absolute (F=71.7, $p<0.001$) and scaled to pronotum length (F=60.77, $p<0.001$), with North clades having longer plates than wētā from South and Central clades (Fig. 3.8). Within North lineage there was much variation in body size with North C significantly larger than North A in most body parts.

Table 3.3. Variation in male terminalia, patterning of the pronotum and numbers of spines and pegs within the *H. maculifrons* species complex.

Clade	n (males & females)	Male SG; male T9 lobes	Pronotum		Mean metatibial spines			Stridulatory pegs			
			Lateral	Dorsal	Prolateral fixed	Retrolateral fixed	Inferior articulated	Femur	T1	T2	T3
Central	22	Flat; pointed	large	Yes	13.61 ±1.35	12.75 ±1.08	1.02 ±0.10	23.00 ±12.96	33.50 ±9.95	40.55 ±10.18	37.77 ±10.85
North A	29	"U"; sinuous	Small	Yes	15.74 ±1.21	15.50 ±0.94	1.16 ±0.32	5.55 ±6.38	46.62 ±14.90	48.62 ±11.31	39.31 ±10.82
North B	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
North C	15	"U" or "V"; sinuous	Small	Yes and no	13.97 ±1.53	13.33 ±1.39	1.10 ±0.20	2.60 ±4.03	36.53 ±7.77	39.87 ±10.27	33.47 ±5.99
North D	3	NA	Small	No	13.50 ±0.41	13.00 ±0.41	1.00 ±0.00	8.33 ±6.55	45.67 ±4.99	47.00 ±2.16	39.67 ±0.47
South A	9	Flat; pointed	Large	Yes	14.50 ±1.31	13.78 ±1.40	2.33 ±0.67	32.56 ±16.13	30.56 ±9.23	37.33 ±10.60	35.56 ±10.75
South B	4	NA	Large	Yes	15.00 ±0.71	13.63 ±0.41	2.13 ±0.22	0.00 ±0.00	22.00 ±12.08	43.25 ±12.07	34.75 ±7.33
South C	28	Flat; pointed	Large	Yes	13.30 ±1.03	12.23 ±1.09	2.00 ±0.00	33.14 ±8.58	27.57 ±6.04	36.75 ±7.62	33.79 ±8.07
South D	1	NA	Large	Yes	14.50	13.50	3.00	43.00	42.00	59.00	42.00
Among clades: Kruskal-Wallis ANOVA χ^2 value ($p<0.005$ for all but T3 ($p>0.05$))					44.90	63.3	90.09	77.86	36.09	20.12	8.83
Among lineages: Kruskal-Wallis ANOVA χ^2 value ($p<0.005$ for all but T3 ($p>0.05$))					20.30	36.20	90.36	58.10	30.42	11.93	3.48

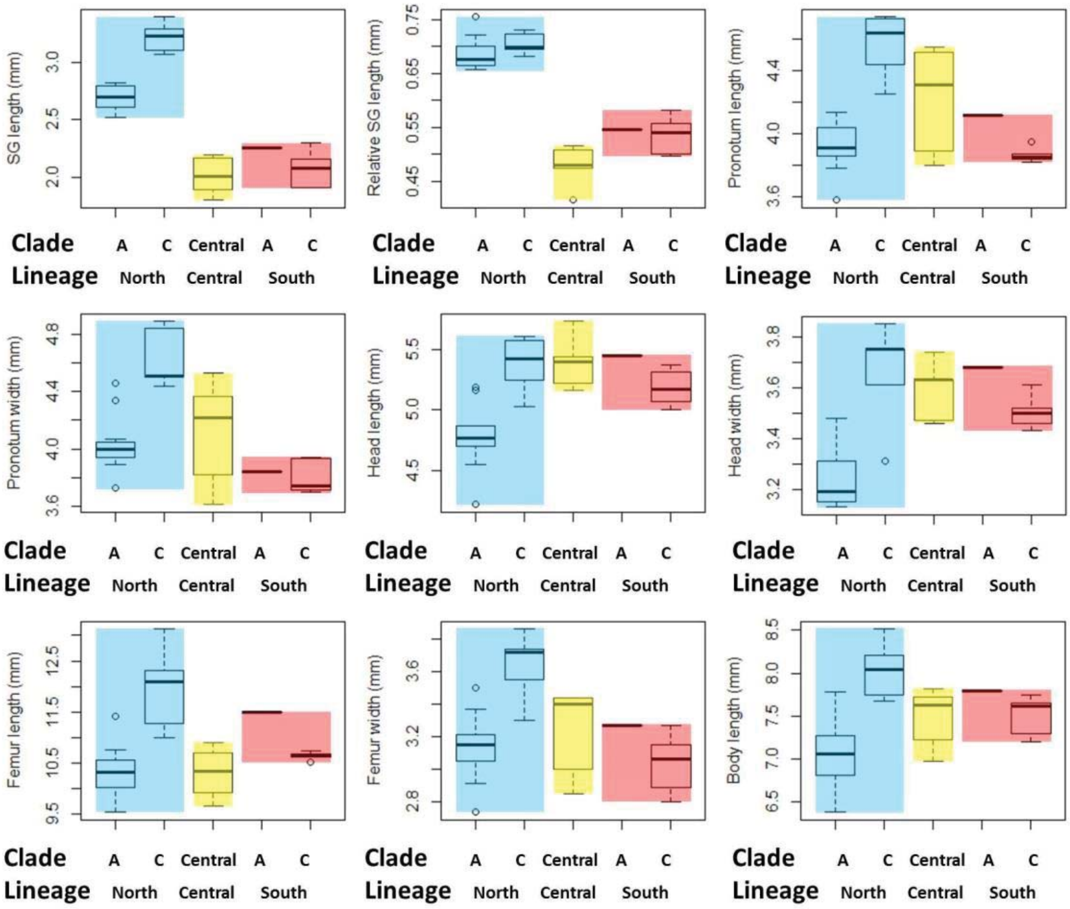


Figure 3.8. Morphometric traits that differed significantly among male *Hemiandrus maculifrons* in different mtDNA lineages and clades. Five clades were included in this analysis: North A, North C, Central, South A, South C. The three main lineages within which the clades were nested are highlighted: North (blue), Central (yellow), South (red). The three main lineages overlap in most traits but the length of the subgenital plate (relative and absolute) clearly separates North lineage from South and Central lineages. Within North lineage, individual males with mtDNA from clade C were larger than those with clade A mtDNA.

Females of the eight clades analysed differed significantly in only four measures: femur length, femur width and ovipositor length (relative and absolute) ($p \leq 0.05$) (Fig. 3.9). However, when comparing the three main lineages, femur length did not differ between lineages, but pronotum length, head length and pronotum width also differed significantly. The most significant difference was in ovipositor length: South lineage had longer ovipositors than North and Central lineages. As for males, North lineage females had high variation: wētā with North C haplotypes were larger than North A in most measurements. The size difference between North A and C may reflect geographical variation as within North lineage females there was a significant positive relationship between all size measurements and longitude (Table 3.5). Only some size measurements were weakly correlated with latitude.

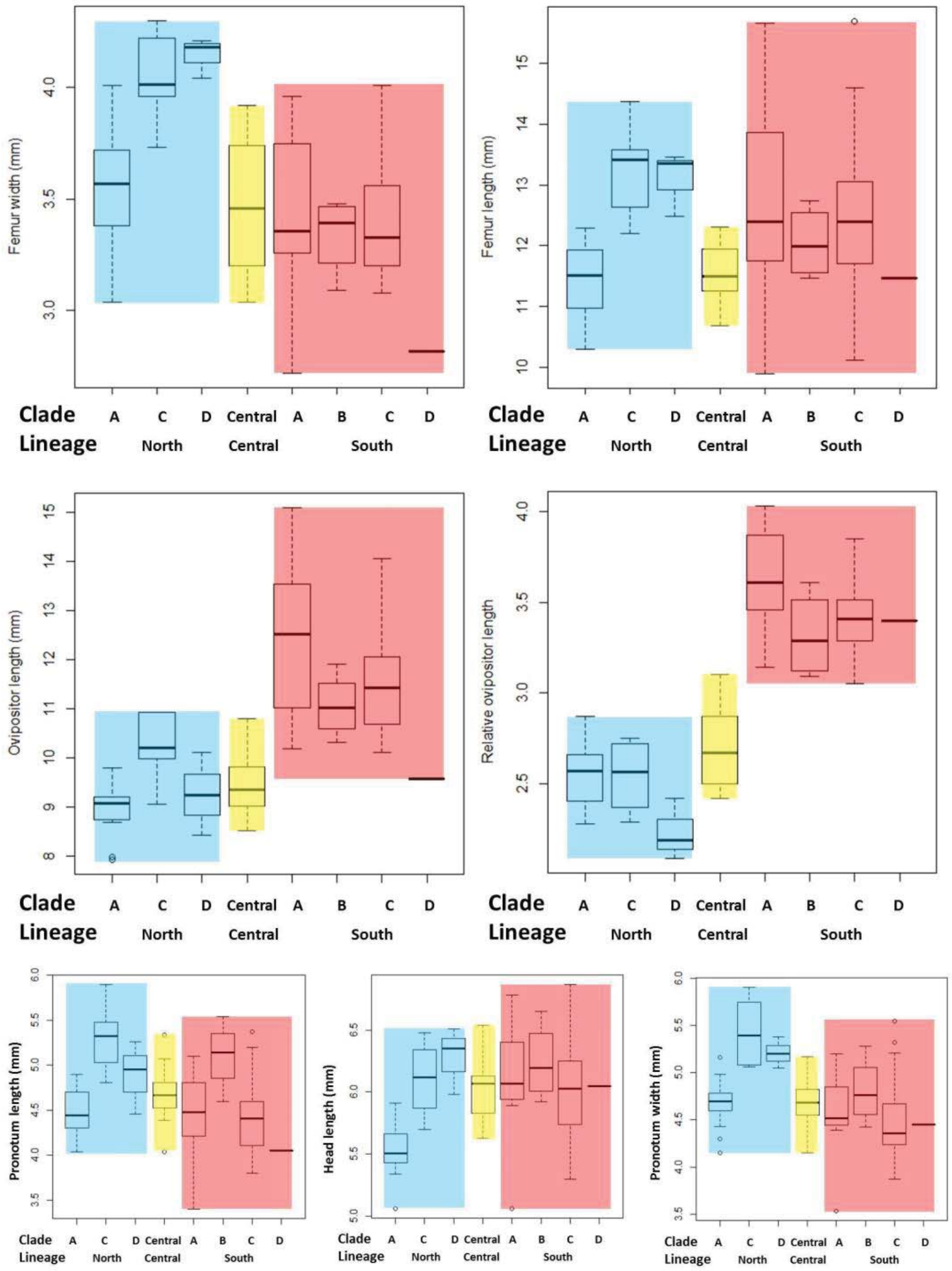


Figure 3.9. Morphometric traits that differed significantly among female *Hemiandrus maculifrons* in different mtDNA lineages and clades. Femur width, femur length and ovipositor length (relative and absolute) differed significantly between clades while femur width, ovipositor length (relative and absolute), pronotum length, head length and pronotum width differed significantly between the three main lineages. Eight of the nine clades were analysed with only North B excluded due to a lack of adult specimens. The three main lineages within which the clades were nested are highlighted: North (blue), Central (yellow), South (red). The 3 main lineages overlap in most traits but relative ovipositor length differs between North and South lineages, while Central overlaps slightly with South lineage.

Table 3.4. One-way ANOVA of metrics of male and female *Hemiandrus maculifrons*. In males, each of the characters differed significantly between individuals in separate mtDNA clades. All measurements in mm.

					Males					
Clade	N	PL	FL	BL	FW	HL	HW	PW	SG	Relative SG
North C	5	4.56±0.19	11.96±0.76	8.04±0.31	3.63±0.19	5.38±0.21	3.65±0.19	4.64±0.19	3.22±0.12	0.71±0.02
North A	13	3.93±0.14	10.30±0.49	7.07±0.39	3.14±0.19	4.78±0.24	3.24±0.11	4.03±0.18	2.69±0.10	0.69±0.03
Central	5	4.21±0.31	10.31±0.47	7.48±0.32	3.22±0.25	5.39±0.20	3.59±0.11	4.11±0.34	2.01±0.15	0.48±0.04
South A	1	4.12	11.49	7.79	3.27	5.45	3.68	3.84	2.25	0.55
South C	5	3.87±0.04	10.65±0.07	7.50±0.21	3.03±0.17	5.18±0.14	3.50±0.06	3.80±0.11	2.07±0.15	0.54±0.03
Anova: 5 clades (df=4)		F=11.18, p≤0.001	F=9.72, p≤0.001	F=7.08, p≤0.001	F=6.14, p=0.002	F=11.01, p≤0.001	F=13.24, p≤0.001	F=9.63, p≤0.001	F=60.77, p≤0.001	F=71.7, p≤0.001
Anova: 3 lineages (df=2)		F=1.46, p=0.25	F=0.63, p=0.54	F=0.46, p=0.63	F=1.16, p=0.33	F=4.72, p=0.02	F=3.50, p=0.05	F=3.47, p=0.05	F=37.18, p≤0.001	F=127.1, p≤0.001
					Females					
Clade	N	PL	FL	BL	FW	HL	HW	PW	OV	Relative OV
North A	16	4.47±0.24	11.42±0.65	7.98±0.63	3.53±0.28	5.53±0.19	3.77±0.19	4.68±0.24	8.97±0.49	2.55±0.19
North C	10	5.30±0.32	13.18±0.67	9.30±0.69	4.04±0.17	6.11±0.26	4.32±0.23	5.41±0.32	10.25±0.61	2.54±0.17
North D	3	4.89±0.33	13.10±0.43	9.95±0.47	4.14±0.07	6.28±0.22	4.28±0.12	5.21±0.13	9.26±0.68	2.23±0.14
Central	17	4.69±0.29	11.57±0.45	8.60±0.79	3.47±0.28	6.05±0.24	4.11±0.13	4.66±0.29	9.42±0.64	2.73±0.23
South A	8	4.44±0.50	12.70±1.64	8.74±0.99	3.43±0.37	6.08±0.48	4.06±0.34	4.54±0.46	12.43±1.58	3.63±0.27
South B	4	5.11±0.33	12.05±0.52	9.26±0.46	3.34±0.15	6.24±0.27	4.14±0.20	4.81±0.31	11.06±0.57	3.32±0.21
South C	23	4.42±0.38	12.43±1.23	8.57±0.84	3.40±0.24	6.01±0.38	4.04±0.31	4.51±0.41	11.59±1.13	3.41±0.16
South D	1	4.05	11.47	9.4	2.82	6.05	3.93	4.45	9.58	3.4
Anova: 8 clades (df=1)		F=3.68, P=0.06	F=6.97, P=0.01	F=1.09, P=0.30	F=4.61, P=0.03	F=3.44. P=0.07	F=0.14, P=0.71	F=3.75, P=0.06	F=62.45, p≤0.0001	F=95.48, p≤0.0001
Anova: 3 lineages (df=1)		F=8.07, p=0.006	F=0.80, p=0.37	F=0.10, p=0.75	F=23.56, p≤0.001	F=6.96, p=0.01	F=0.22, p=0.64	F=18.29, p≤0.001	F=63.76, p≤0.001	F=243, p≤0.001

Table 3.5. The pooled body sizes of North A and North C females were significantly correlated with longitude. Only some were correlated with latitude.

Variable	longitude		latitude	
	p	R	p	R
FL	0.0004	0.65	≥0.05	0.38
OV	0.0002	0.66	≥0.05	0.25
BL	0.02	0.45	0.03	0.43
FW	0.0004	0.55	0.04	0.40
HL	0.0001	0.68	0.03	0.43
HW	0.0003	0.66	≥0.05	0.31
PW	0.0004	0.64	0.01	0.49
PL	0.0003	0.65	0.04	0.41

Principal component analysis (PCA) of male wētā showed that the first two components explained 83% (56.43% and 26.49%) of the total variance (Fig. 3.10B). All characters had positive loadings on PC1 and the highest loadings were associated with the length and width of the pronotum and femur (Appendix 3.3). The highest (and positive) loadings on PC2 were the relative and absolute length of the subgenital plate. PCA supported the separation of North clade from all others based on PC2 (i.e. subgenital plate length) and the separation of North A and North C based on PC1 (i.e. body size). For female wētā PC1 and PC2 explained 87% (61.83% and 25.15%) of the total variance (Fig. 3.10A). All variables loaded positively on PC1 except for relative ovipositor length (Appendix 3.3). The highest loadings on PC1 were femur length

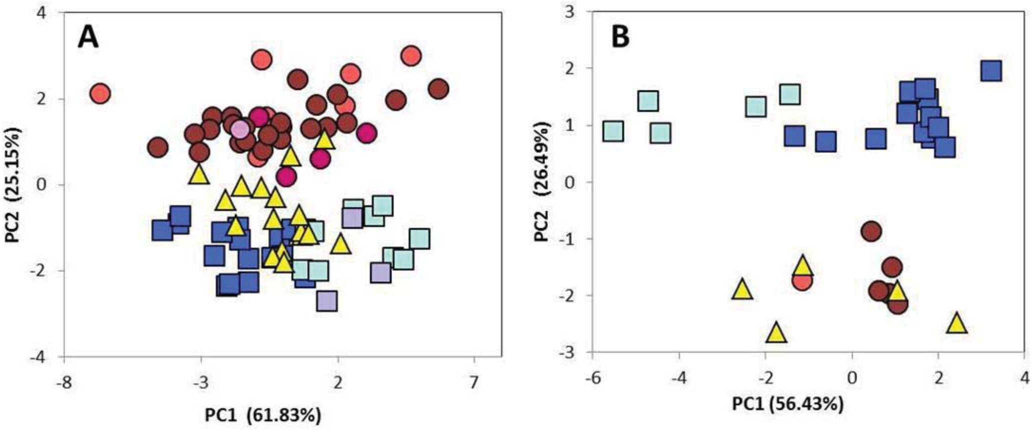


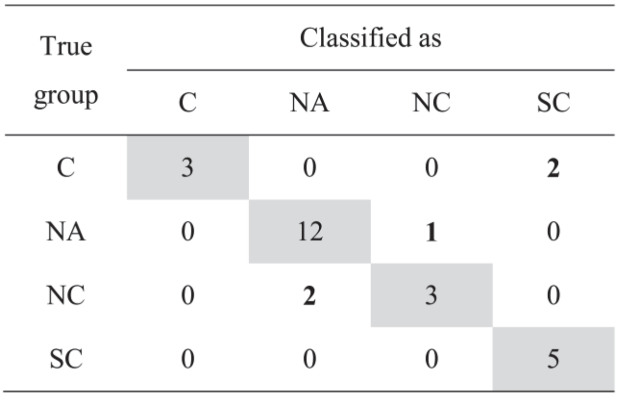
Figure 3.10. Principal component analysis of *H. maculifrons* body size measurements. **A.** In females, PC1 (body size) separated North A from North C and North D females while PC2 (ovipositor length) separated North lineage females from South females. **B.** In males PC1 (body size) separated North A from North C while PC2 (subgenital plate length) separated North lineage from South lineage individuals. Squares represent North lineage, triangles represent Central, circles represent South lineage. Colours represent clades as in Figure 3.5.

and head width. PC1 largely separated North A from North C and North D females. Relative ovipositor length had the highest loading on PC2 and separated North from South females.

“Gaps in Continuous Characters across Geography” analysis was applied to male and female PC1 and PC2 scores. For males there was evidence of bimodality between North A and North C individuals and all others. For females there was evidence of bimodality between South and North, between South and Central, between South A and all other South and between North A and North C. However in all cases, no significant discontinuity was present suggesting that all the morphological variation present within the data represents intraspecific variation (Zapata and Jimenez 2012).

A linear discriminant function analysis (DFA) with cross validation was used to determine whether the measurement data could predict clade membership. DFA of adult males representing five clades of *H. maculifrons* resulted in 92.31% correct classification (Table 3.6), but South and Central clades were confounded. Within North, both clades tested were confounded with one another but not with South and Central lineages. The DFA on females correctly classified 70.37% of individuals to each of seven clades (Table 3.7). As with males, South and central were confounded with each other, but North clades and Central were also confounded with one another. Female metrics had less discriminating power than those of males.

Table 3.6. Discriminant function analysis with cross validation of continuous morphological traits in male *H. maculifrons* resulted in 92.31% correct clade assignment (grey). Incorrect assignments are in bold text.



True group	Classified as			
	C	NA	NC	SC
C	3	0	0	**2**
NA	0	12	**1**	0
NC	0	**2**	3	0
SC	0	0	0	5

Table 3.7. Discriminant function analysis with cross validation of continuous morphological traits in female *H. maculifrons* resulted in 70.37% correct clade assignment (grey). Incorrect assignments are in bold text.

True group	Classified as						
	C	NA	NC	ND	SA	SB	SC
C	15	0	0	0	0	**2**	0
NA	0	14	**1**	**1**	0	0	0
NC	**2**	**3**	5	0	0	0	0
ND	0	0	**3**	0	0	0	0
SA	1	0	0	0	1	**1**	**5**
SB	1	0	0	0	0	1	**2**
SC	0	0	0	0	**2**	0	21

A paired *t*-test indicated that male and female wētā did not differ in their numbers of spines and pegs so these were analysed together (Table 3.3). A Kruskal-Wallis one-way ANOVA indicated that spine and peg numbers differed between wētā in different mtDNA clades and between the three main lineages, except for T3 pegs. The largest differences were in the number of inferior mid tibial spines and hind femur pegs (Fig. 3.11). South lineage wētā had significantly more metatibial pegs than the North and Central clades. In fact all individuals within the South lineage had two or more spines on each metatibia, while 21 of 22 central clade individuals had a single spine on each leg. A single Central individual, which was not found at a site where other mtDNA lineages were present, had two on one leg and one of the other. Wētā in the North lineage had fewer femoral pegs than those in Central and most South clades, although all four South B individuals had no femoral pegs.

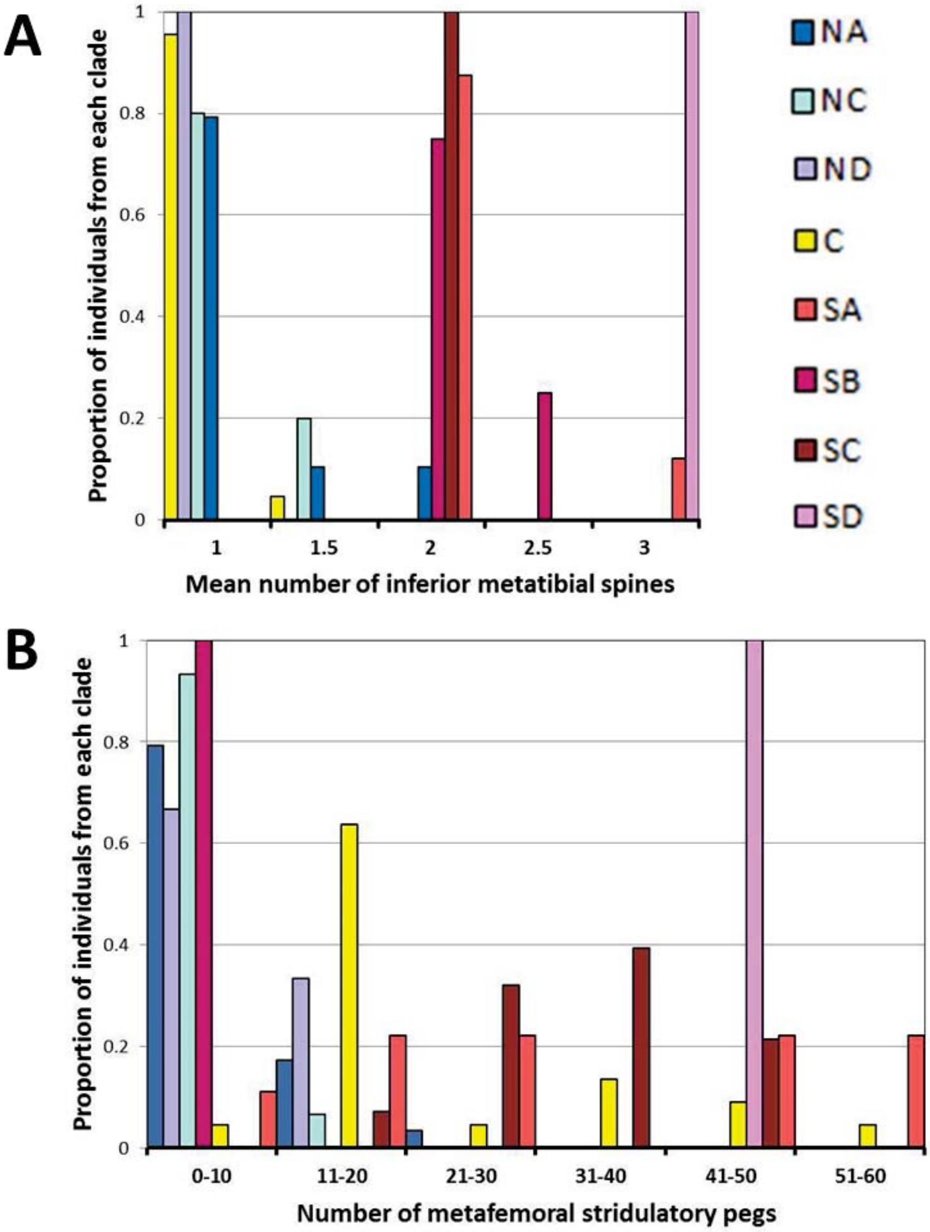


Figure 3.11. Variation in spine and peg number among *Hemiandrus maculifrons* mtDNA clades. Differences among clades were most apparent in the number of inferior metatibial spines (**A**) and pegs of the hind femur (**B**).

Geometric morphometric analyses

Geometric morphometric analysis of ovipositor shape included data from 76 females representing eight clades and all three lineages. The first two principal components accounted for 84.17% of the variation (Fig. 3.12). Although elliptic Fourier analysis removes any size effects, PC1 approximates to the width of the ovipositor, and was strongly negatively correlated with ovipositor length ($R^2=0.84$) indicating that shorter ovipositors tend to be stouter. PC2 was associated with curvature of the ovipositor, with positive values being more curved. Ovipositors measured of South lineage wētā tended to be straight and slender compared to those of other clades which were more curved and broader. Central lineage was separated by PC1 from most South clades but not South D. Although there was clustering of individuals belonging to the same clade, there was no complete separation. “Gaps in Continuous Characters across Geography” analysis supported the presence of bimodality between South and Central clades and most North versus South clades. In all instances a tolerance region covering proportions β < 0.9 overlapped failing to support the hypothesis of a species boundary. South D was not included in GCCG.

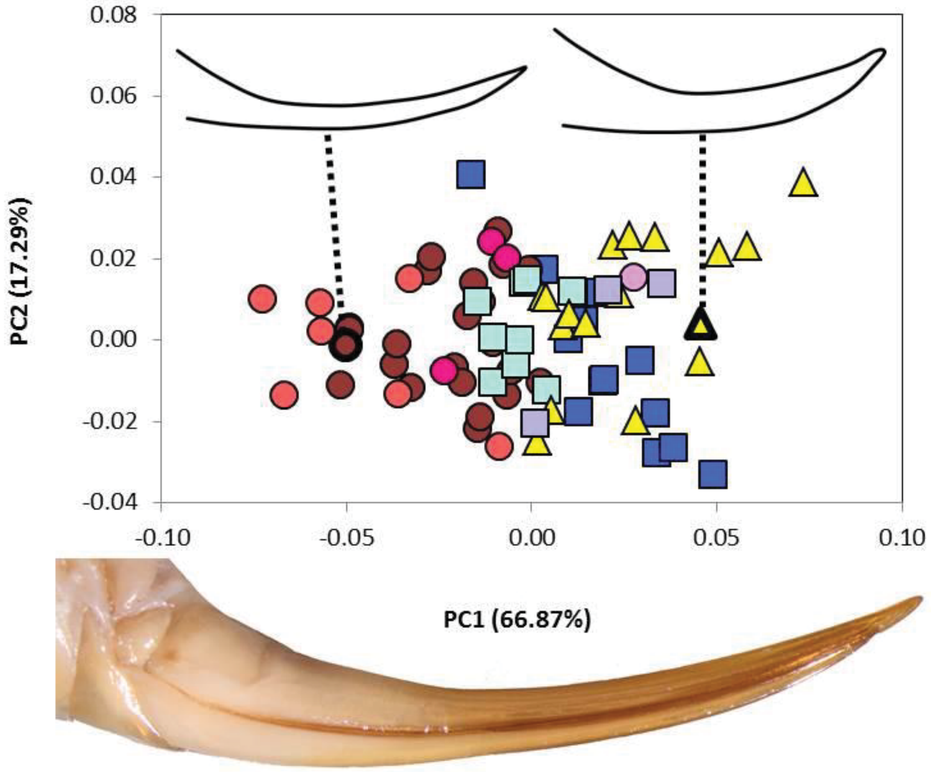


Figure 3.12. Principal component analysis of female ovipositor shape. The outlines show the shape associated with PC1 values of approximately -0.05 and 0.05 respectively, where PC2 is approximately zero. Below is an ovipositor photographed in lateral view. PC1 separated South and Central lineages apart from a single South lineage (FD4, South D) falling within the cluster of Central specimens.

The relative positioning of mesotibial spines was analysed for 67 specimens representing seven mtDNA clades. The first two PC principal components accounted for 74.06% of the variance. PC1 was associated with the symmetry of the spines, with positive values being less symmetrical (Fig. 3.13). PC2 was associated with the positioning of the middle spines relative to the upper and lower spines. South and Central lineages had spines that were more positioned more symmetrically than those of North lineage wētā. GCCG detected bimodality between North clades and others but there was an overlap in tolerance regions indicating that there was no significant morphological gap.

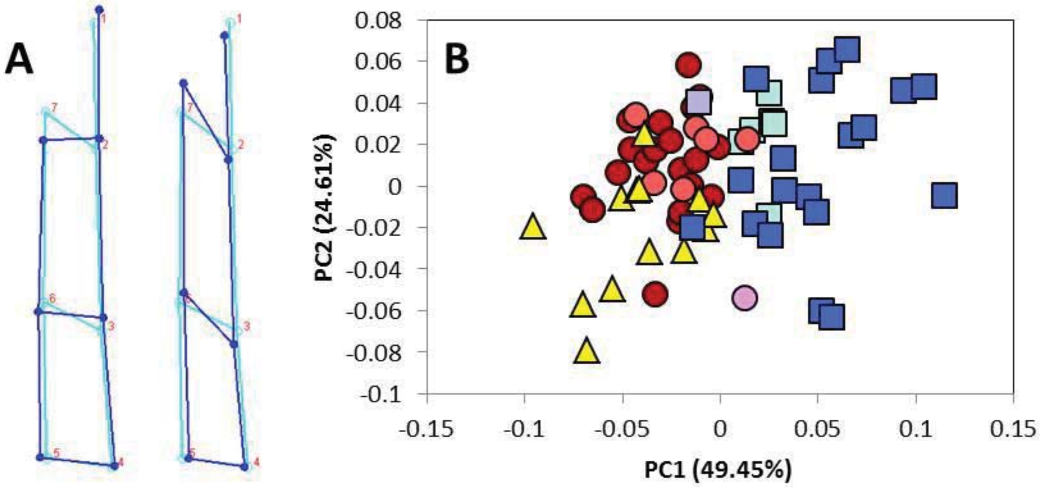


Figure 3.13. **A.** The relative position of midtibial spines in *Hemiandrus maculifrons*. **A.** Landmarks used for geometric morphometric analysis. The points indicate the position of the spines while the lines help to illustrate the change in their relative position. The dark blue outlines show the shape change associated with PC1: to the left is PC1 with a scale factor of -0.09, right is 0.09. The pale blue outlines show a scale factor of zero. **B.** PCA of the relative position of spines. Included in the analysis were 67 individuals representing 7 clades. South lineage (circles) and Central lineage (triangles) tended to have more symmetrically positioned spines, while North lineage (squares) had spines positioned more asymmetrically.

Summary of results

Mitochondrial DNA sequencing of *Hemiandrus maculifrons* specimens collected from throughout New Zealand detected high diversity with pairwise genetic distances exceeding that typically expected for intraspecific variation (uncorrected =17%). Phylogenetic analysis of the mtDNA resolved nine clades nested within three main lineages that formed hypotheses to test putative specific status. Three nuclear markers revealed fixed differences between North lineage wētā and those wētā with South or Central mtDNA haplotypes. Where sympatric, South and Central mtDNA wētā also had distinct nuclear alleles. Morphological characters also clearly differentiated wētā with the North lineage mtDNA haplotypes from all others. Although fewer morphological characters differentiated South and Central mtDNA lineages, diagnostic spines differences were observed as well as quantitative differences. In contrast, the mtDNA subclades within lineages did not resolve morphologically definable units.

Table 3.8. Summary of genetic and morphometric evidence for subgroups within the widespread New Zealand ground wētā, *Hemiandrus maculifrons*.

Lineage	Clade	Test						
		Sympatry	Geneious species delimitation	Bayesian species delimitation	Private nuclear alleles	Mean inferior metatibial spines	Relative OV length	Male terminalia
North	**North A**	North D, Central	Y	Y	Y	≤2	Short	Long SG with "V" or "U"-shaped edge, sinuous T9 edge
	North B	No	Y	Y	Y			
	North C	Central	Y	NA	NA			
	North D	North A	Y	NA	Y			
Central	Central	North A, North C, South A, South C	Y	Y	Y	≤1.5	Short	Short SG with flat edge, pointed T9 lobes
South	South A	Central, South C	Y	Y	Y	≥2	Long	Short SG with flat edge, pointed T9 lobes
	South B	No	Y	NA				
	South C	South A, Central	Y	Y				
	South D	No	No (PID <93)	NA				

Discussion

***Hemiandrus maculifrons* is a species complex**

Hemiandrus maculifrons, as currently recognised, has an unusually large geographic range compared to other ground wētā species. I found that the range of *H. maculifrons* is more widespread than previously recognised but it appears to be restricted to primary native forest. The existence of morphological differences between wētā with very different mtDNA haplotypes that are maintained in sympatry indicates that rather than being a single widespread species with high population differentiation *H. maculifrons* is better treated as comprising numerous independent evolutionary lineages.

MtDNA sequence divergence within the sample was very high (23% K2P-corrected genetic distance). High genetic distances within a species might be due to sustained large population sizes and demographic stability. New Zealand wētā are known to have genetic diversity above typical levels of mtDNA divergence among insect species e.g. more than 9% uncorrected in *Hemideina thoracica* (Morgan-Richards *et al.* 2001), 9% uncorrected in *Hemiandrus pallitarsis* (Chappell *et al.* 2012), 8% K2P corrected in *Deinacrida connectens* (Trewick *et al.* 2000; Trewick 2001). The diversity within *H. maculifrons*, however, exceeds that of other wētā species. In contrast, the maximum diversity within each of the three main *H. maculifrons* mtDNA lineages, 9.1-9.7% (uncorrected), approximates intraspecific distances observed within other widespread New Zealand Anostostomatids.

Except for a single individual from northwest Nelson, which clustered with no other individuals, *H. maculifrons* comprised nine clades that were well-supported in both Bayesian and ML phylogenetic analyses. All clades tested were supported by Bayesian species delimitation as separate species, and all but one (South D) was supported by most species delimitation statistics based on mtDNA evidence. Six of the nine clades were found to be sympatric with at least one other clade (Table 3.8). The nine clades nested within three main mtDNA lineages and concordance with nuclear markers was found. South and Central lineages and all North clades for which nuclear loci were available had some private alleles. Private alleles indicate a lack of gene flow between lineages or species at that locus. Sharing of other alleles between lineages could be due to incomplete lineage sorting or gene flow. However, fixed differences occurring in sympatry are indicative of reproductive isolation and thus independent evolutionary trajectories. Wētā representing the North mtDNA lineage shared no nuclear alleles with Central lineages even at adjacent locations. Although some nuclear alleles were shared between wētā representing South and Central mtDNA lineages, at Lewis pass alternative alleles were fixed at two loci (Table 3.2). Thus the nuclear loci provide strong support for assortative mating where wētā from different mtDNA lineages co-occur.

Morphological traits (male terminalia size and shape, ovipositor length and inferior metatibial spines) were concordant with the three main mtDNA lineages, but within some lineages there was a high degree of morphological variation. *H. maculifrons* appears to consist of three distinct morphological and genetic clusters that appear to have no intermediates when Central lineage and North lineage come into contact on North Island and although a nuclear allele is shared between Central and South lineage in sympatry on South Island, there is no introgression of mtDNA and morphology is fixed between lineages. Therefore, applying the genotypic cluster approach (Mallet 1995), *H. maculifrons* is best treated as consisting of three species. The fact that some species delimitation methods recognised *H. maculifrons* as comprising fewer than or more than three species may be due to the high level of information content in mtDNA relative to nuDNA (Bayesian species delimitation) and assumptions of the model such as random mating ((P(Randomly distinct), P(AB)). Increased sampling of adults from all nine mtDNA clades might resolve additional genotypic clusters but current data confirms three species.

North species expressed a high degree of variation in body size, patterning of the pronotum and subgenital plate shape, although the latter two traits were found to vary even within a single population (North C) suggesting that they are phenotypically plastic. Subgenital plate variation could alternatively be the result of maturation of males at different instars as has been recorded in *Hemideina crassidens* (Spencer 1995), but previously all South lineage *H. maculifrons* males were found to mature at the 8th instar (Cary 1981). Absolute body size measurements differed between North A and North C with specimens in North C larger than those forming North A, resulting in bimodality of the two clades in the PCA (Fig. 3.10). All size variables were, however, correlated with longitude (Table 3.5), suggesting that size differences could be a response to geographically varying environmental conditions. Furthermore, when subgenital plate and ovipositor length were scaled to body size there were no significant differences between North A and North C suggesting that although size differs, body proportions remain the same. These two clades were not found in sympatry so the persistence of size variation independent of location could not be assessed. I found morphological differences between North A and North D clades, which were sympatric at Pureora Forest Park. Although pronotum patterning varied between North A and North D, sample size is too small (n=3) to draw conclusions. Currently there is insufficient data to test whether North D and North A are separately evolving lineages. Each North clade genotyped (A, B, D) had some private nuclear alleles as expected of interspecific variation, but it is not currently possible to distinguish this from a single species with high population differentiation and isolation by distance because sample sizes are small and/or lack of sympatry.

Genotypic clusters

One of the advantages of the genotypic cluster definition of species (Mallet 1995) is it does not rely on interbreeding or the lack of it. Where the three species are in contact with one another introgression has not been detected. North species has male terminalia differences that distinguish it from South and Central species. Male terminalia often rapidly diverges between species as the result of sexual selection (Arnqvist 1998; Hosken and Stockley 2004). Terminalia differences can result in prezygotic isolation between species. The apparent absence of terminalia differences between South and Central indicates that other isolating mechanisms are likely present. Where South and Central occur together (Lewis Pass) assortative mating is evident in all traits examined (genetic loci, size, shape and spines). So although physical barriers to interbreeding have not been detected something must limit the production of intermediates. Variation in male terminalia within North suggests that some differences in terminalia may not necessarily limit gene flow. In other New Zealand *Hemiandrus* courtship drumming is thought to be important for successful copulation (Gwynne 2004) and so interspecific differences in this behaviour may play a role in limiting gene flow between some species.

Phylogeography

Closely related species that have allopatric or parapatric ranges can provide useful insights into the processes that shape the partitioning and distribution of biodiversity. If species ranges overlap completely then both patterns and processes may be obscured. Species of the *H. maculifrons* complex are allopatric or parapatric, suggesting an allopatric origin. The sympatry of these species only on the edges of their ranges suggests that the species may be being held apart by ecological competition, although they may be in the process of merging their ranges.

Central and North species were sympatric at two sites in North Island. Central species appears to be found only on the axial ranges, whereas North species was found also in lowland forest. At the two sites where these two clades were found in sympatry, Central was found at higher altitudes than North species. This distributional pattern is similar to that seen in tree wētā *Hemideina thoracica* and *H. crassidens*. Like North species, *H. thoracica* has a more northerly distribution than *H. crassidens*, which has a more southerly distribution somewhat similar to Central. The two tree wētā species are sympatric at some sites on North Island where *H. crassidens* populations are marooned at colder higher altitudes, possibly due to competitive exclusion from *H. thoracica* who is adapted to warmer habitats (Bulgarella *et al.* 2014; Trewick and Morgan-Richards 1995). In some sites where these two species meet in sympatry, low levels of introgression have been detected whereas niche divergence and sterile F1 hybrids are present where *H. thoracica* is found in sympatry with another tree wētā species (*H. trewicki*) (McKean *et al.* In Press). Thus the effects of secondary contact are varied, and the exact nature of interactions between species within the *H. maculifrons* species complex are yet to be determined. Now that the species diversity within this complex has been recognised, further sampling will allow more explicit tests of the nature of interspecific interactions and drivers of range size and species distributions.

Central and South species were sympatric at Lewis Pass in South Island. This location delimits the currently know distribution of three mtDNA clades with South A and Central clades found north of this point and South C found south of Lewis Pass. The distribution of these two South mtDNA clades is similar to that of *Tallitropsis sedilloti* which was attributed to post glaciation range expansion (Trewick and Wallis 2001). The other two South mtDNA clades (D & B) are restricted to southern South Island where they may have existed in refugia during glacial periods. If glaciation has restricted the southern boundary of South A, then it may also have restricted the distribution of Central. Alternatively, the range of Central in South Island may be restricted by competition with South species.

Conclusions

Range size is influenced by many biotic and abiotic factors. Rather than being an unusually widespread species, the results presented here suggest that *H. maculifrons* is a cryptic species complex. Species are difficult to define and species delimitation is controversial, however, the incorporation of multiple lines of evidence is important for constructing robust taxonomic hypotheses as any single line of evidence can be misleading. The concordance of evidence in this study suggests that *H. maculifrons* is best treated as three species. However, the application of different species concepts and methods may result in the recognition of more species diversity.

Appendix

Appendix 3.1. Species delimitation methods

Bayesian species delimitation

A multilocus, coalescent species delimitation analysis was conducted using the program BPP 2.2 (Rannala and Yang 2003, 2013; Yang and Rannala 2010). This method adopts the biological species concept (BSC) (see Yang and Rannala (2010)), and using a species phylogeny represented by a user-specified guide tree, it accommodates lineage sorting because of ancestral polymorphism. The mtDNA tree was used as the guide tree, although only 5 clades had all 4 loci available and could be included in the analysis. A gamma prior (G) was used to specify the population size parameter θ and root age τ_0 of the species tree. Three population size/species tree age combinations were modelled (e.g. see (Leaché and Fujita 2010)): large *Ne* + deep divergence time [$G(1,10)$ for θ and τ_0], large *Ne* + shallow divergence time [$G(1,10)$ for θ and $G(2,2000)$ for τ_0] and small N_e + shallow divergence time [$G(2,2000)$ for θ and τ_0]. Other divergence time parameters were assigned a Dirichlet prior (Yang and Rannala 2010: equation 2). Each analysis of 500 000 MCMC generations was run twice from different starting seeds with a burn-in period of 50 000; this gave consistent parameter estimates between replicate runs and ESS values >1000 for all parameters.

Gaps in continuous characters across geography (GCCG)

Most morphological statistical analyses examine differences in central tendency whereas Zapata and Jimenez (2012) developed a test for assessing the strength of evidence indicating a gap or discontinuity in continuous multivariate morphological data. The theory behind using a gap is that morphological variation in a population with random mating is described by a normal distribution (Sokal and Rohlf 1995; Templeton 2006), whereas if there are two species then morphological variation should be described by two normal distributions and thus two modes separated by a gap (Futuyma 1998) (under the assumption that multimodal distributions are not the result of polymorphisms, ontogenetic variation or phenotypic plasticity).

To assess the number of modes in multivariate space X, the first step in this method was to carry out a PCA of characters. These scores were then used to calculate the probability density function (pdf) of the multivariate morphological space (f(X)). Also calculated was the ridgeline manifold which is a curve that includes all critical points (maxima, minima, and saddles) of f(X). Points along the ridgeline manifold correspond to values of variable α that range from zero at the multivariate mean of one hypothesized species to one at multivariate mean of the other. A plot of the pdf along the ridgeline manifold was inspected for the presence of more than a single mode. If the graph showed bimodality then a further test of how distinct the two hypothesized species were was carried out. Using an *a priori* frequency cut-off of 0.1 i.e. β=0.90 (γ =0.95), below which overlap of phenotypic overlap was considered to be rare enough to suggest that the two lineages were isolated, univariate tolerance regions for normal distributions were assessed to determine the degree of phenotypic overlap between hypothesized species. If a significant gap was detected, then the next step would be to perform a redundancy analysis to test whether this gap was due to geographic variation or a species boundary.

Appendix 3.2. *Hemiandrus maculifrons* specimens used in the analysis. Sex is specified for specimens used in the morphological analysis.

Code	COI 740 bp	COI 501 bp	mtDNA lineage	Sex	NU 2	NU 3	NU 13	Latitude	Longitude	Location
GW23	x	Y	Central	F	Y	x	Y	-42.3141	172.1177	Lewis Pass
GW143	Y	Y	Central	F	x	x	x	-42.3141	172.1177	Lewis Pass
GW1013	Y	Y	Central	F	x	Y	Y	-42.3141	172.1177	Lewis Pass
GW1014	Y	Y	Central	F	Y	Y	Y	-42.3141	172.1177	Lewis Pass
GW1015	Y	Y	Central	F	Y	Y	Y	-42.3141	172.1177	Lewis Pass
GW900	Y	Y	Central	F	Y	Y	Y	-40.9346	172.9394	Abel Tasman NP
GW568	Y	Y	Central	F	Y	Y	Y	-40.9265	172.8576	Rameka
GW574	Y	Y	Central	F	Y	Y	Y	-40.9265	172.8576	Rameka
GW571	Y	Y	Central	F	x	x	x	-40.9265	172.8576	Rameka
GW100	x	Y	Central	F	x	x	x	-40.9265	172.8576	Rameka
GW105	x	Y	Central	F	x	x	x	-40.9265	172.8576	Rameka
GW761	Y	Y	Central	F	x	x	x	-40.4857	175.6367	Kahuterawa
GW815	Y	Y	Central	F	x	x	x	-40.4857	175.6367	Kahuterawa
GW817	Y	Y	Central	F	x	x	x	-40.4857	175.6367	Kahuterawa
GW916B	Y	Y	Central	F	x	x	x	-39.8111	176.1354	Ruahine FP
GW553	Y	Y	Central	F	Y	Y	Y	-39.6772	176.2501	Ruahine FP
GW552	Y	Y	Central	F	Y	Y	Y	-39.6730	176.2122	Ruahine FP
GW1010	Y	Y	Central	M	Y	Y	Y	-42.3141	172.1177	Lewis Pass
GW1012	x	Y	Central	M	x	Y	Y	-42.3141	172.1177	Lewis Pass
GW573	Y	Y	Central	M	x	x	x	-40.9265	172.8576	Rameka
GW104	Y	Y	Central	M	x	x	x	-40.9265	172.8576	Rameka
GW759	Y	Y	Central	M	x	Y	Y	-40.4857	175.6367	Kahuterawa
GW611	Y	Y	Central	x	Y	x	Y	-40.6921	175.5233	Tararua FP
GW711	Y	Y	Central	x	x	x	x	-40.4857	175.6367	Kahuterawa
GW816	x	Y	Central	x	x	x	x	-40.4857	175.6367	Kahuterawa
GW465	Y	Y	Central	x	Y	x	Y	-38.7664	177.1694	Waikaremoana
GW750	Y	Y	Central	x	x	x	x	-38.7664	177.1694	Waikaremoana
GW752	x	Y	Central	x	x	x	x	-38.7664	177.1694	Waikaremoana
GW819	x	Y	North	F	Y	Y	x	-40.4857	175.6367	Kahuterawa
GW765	Y	Y	North	F	x	x	x	-40.4719	175.6142	Kahuterawa
GW807	Y	Y	North	F	x	x	x	-40.4719	175.6142	Kahuterawa
GW811	Y	Y	North	F	x	x	x	-40.4719	175.6142	Kahuterawa
GW557	Y	Y	North	F	x	x	x	-39.7271	175.1388	Atene
GW893	Y	Y	North	F	x	x	x	-39.3246	174.1065	Mt Taranaki
GW777	Y	Y	North	F	x	x	x	-39.1487	173.9389	Lucy's Gully
GW778	Y	Y	North	F	x	Y	x	-39.1487	173.9389	Lucy's Gully
GW919	Y	Y	North	F	x	x	x	-39.1289	174.1218	Mangamahoe
GW624	Y	Y	North	F	x	x	x	-38.8473	175.5574	Pureora FP
GW628	Y	Y	North	F	x	x	x	-38.8473	175.5574	Pureora FP

GW631	Y	Y	North	F	x	x	x	-38.8473	175.5574	Pureora FP
GW633	Y	Y	North	F	x	x	x	-38.8473	175.5574	Pureora FP
GW196	Y	Y	North	F	x	x	x	-38.7672	176.2187	Opepe
GW195ii	Y	Y	North	F	x	x	x	-38.7672	176.2187	Opepe
GW90	x	Y	North	F	x	x	x	-38.7575	177.1514	Waikaremoana
GW740	Y	Y	North	F	x	x	x	-38.7575	177.1514	Waikaremoana
GW741	Y	Y	North	F	x	x	x	-38.7575	177.1514	Waikaremoana
GW742	Y	Y	North	F	x	x	x	-38.7575	177.1514	Waikaremoana
GW745	Y	Y	North	F	x	x	x	-38.7575	177.1577	Waikaremoana
GW746	x	Y	North	F	x	x	x	-38.7575	177.1577	Waikaremoana
GW736	Y	Y	North	F	x	x	x	-38.7575	177.1514	Waikaremoana
GW126	Y	Y	North	F	x	x	x	-38.5169	175.5807	Pureora FP
GW549	Y	Y	North	F	x	x	x	-38.5169	175.5807	Pureora FP
GW700	Y	Y	North	F	x	x	x	-38.3000	177.3333	Wairata
GW703	Y	Y	North	F	x	x	x	-38.3000	177.3333	Wairata
GW4	x	Y	North	F	x	x	x	-38.2918	177.3848	Manganuku Bridge
GW49a	x	Y	North	F	Y	Y	x	-35.1873	173.7629	Puketi
GW726	x	Y	North	F	x	Y	x	-35.1873	173.7629	Puketi
GW813	Y	Y	North	M	Y	Y	Y	-40.4719	175.6142	Kahuterawa
GW767	Y	Y	North	M	x	x	x	-40.4719	175.6142	Kahuterawa
GW769	Y	Y	North	M	x	x	x	-40.4719	175.6142	Kahuterawa
GW772	Y	Y	North	M	x	x	x	-40.4719	175.6142	Kahuterawa
GW773	Y	Y	North	M	x	x	x	-40.4719	175.6142	Kahuterawa
GW775	Y	Y	North	M	x	x	x	-40.4719	175.6142	Kahuterawa
GW814	Y	Y	North	M	x	x	x	-40.4719	175.6142	Kahuterawa
GW558	Y	Y	North	M	x	x	x	-39.7271	175.1388	Atene
GW222	x	Y	North	M	x	x	x	-39.1720	174.9549	Ohauora
GW225	x	Y	North	M	x	x	x	-39.1720	174.9549	Ohauora
GW218	x	Y	North	M	x	x	x	-39.1241	175.3914	Raurimu
GW625	Y	Y	North	M	x	x	x	-38.8473	175.5574	Pureora FP
GW753	Y	Y	North	M	x	x	x	-38.7664	177.1694	Waikaremoana
GW735	Y	Y	North	M	x	x	x	-38.7575	177.1514	Waikaremoana
GW68	Y	Y	North	M	x	x	x	-38.7575	177.1514	Waikaremoana
GW738	Y	Y	North	M	x	x	x	-38.7575	177.1514	Waikaremoana
GW739	x	Y	North	M	x	x	x	-38.7575	177.1514	Waikaremoana
GW550	Y	Y	North	M	Y	Y	Y	-38.5169	175.5807	Pureora FP
GW1026a	Y	Y	North	x	x	x	x	-41.7590	172.9697	Mt Richmond FP
GW1026b	Y	Y	North	x	x	x	x	-41.7590	172.9697	Mt Richmond FP
GW907	Y	Y	North	x	Y	Y	Y	-41.2969	173.5760	Pelorus Bridge
GW88A	Y	Y	North	x	Y	x	Y	-41.2969	173.5760	Pelorus Bridge
GW897A	Y	Y	North	x	Y	Y	Y	-41.2969	173.5760	Pelorus Bridge
GW897B	Y	Y	North	x	Y	Y	Y	-41.2969	173.5760	Pelorus Bridge
GW93a	x	Y	North	x	x	x	x	-41.2969	173.5760	Pelorus Bridge

GWATENE	Y	Y	North	x	Y	Y	Y	-39.7271	175.1388	Atene
GW238	Y	Y	North	x	x	x	x	-39.1487	173.9389	Lucy's Gully
GW221	Y	Y	North	x	x	x	x	-39.1241	175.3914	Raurimu
GW626	Y	Y	North	x	x	x	x	-38.8473	175.5574	Pureora FP
GW632	Y	Y	North	x	x	x	x	-38.8473	175.5574	Pureora FP
GW109	Y	Y	North	x	x	x	x	-38.7716	177.1430	Opepe
GW737	Y	Y	North	x	x	x	x	-38.7575	177.1514	Waikaremoana
GW701	Y	Y	North	x	x	x	x	-38.3000	177.3333	Wairata
GW551	x	Y	North	x	x	x	x	-38.8473	175.5574	Pureora FP
GW801	x	Y	North	x	x	x	x	-40.4719	175.6142	Kahuterawa
GW119	x	Y	South	F	x	x	x	-46.5721	169.4556	Catlins
GW32A	x	Y	South	F	x	x	x	-46.5721	169.4556	Catlins
GW1099	x	Y	South	F	x	x	x	-46.1806	168.4281	Forest Hill
GW201	Y	Y	South	F	x	x	x	-45.5927	167.9514	Takitimu Range
FD4	Y	Y	South	F	Y	Y	Y	-44.6392	167.8306	Sinbad Gully
GW908	Y	Y	South	F	Y	Y	Y	-42.9462	171.5644	Arthur's Pass
GW912	Y	Y	South	F	x	Y	Y	-42.9462	171.5644	Arthur's Pass
GW898	Y	Y	South	F	x	x	x	-42.9462	171.5644	Arthur's Pass
GW909B	Y	Y	South	F	x	x	x	-42.9462	171.5644	Arthur's Pass
GW910B	Y	Y	South	F	x	x	x	-42.9462	171.5644	Arthur's Pass
GW913A	Y	Y	South	F	x	x	x	-42.9462	171.5644	Arthur's Pass
GW914A	Y	Y	South	F	x	x	x	-42.9462	171.5644	Arthur's Pass
GW915A	Y	Y	South	F	x	x	x	-42.9462	171.5644	Arthur's Pass
GW836	Y	Y	South	F	Y	Y	Y	-42.6479	171.0626	Awatuna
GW837	Y	Y	South	F	Y	Y	Y	-42.6479	171.0626	Awatuna
GW922F	Y	Y	South	F	x	x	x	-42.4693	171.2526	Greymouth
GW924A	Y	Y	South	F	x	x	x	-42.4693	171.2526	Greymouth
GW925A	Y	Y	South	F	x	x	x	-42.4693	171.2526	Greymouth
GW872	Y	Y	South	F	Y	Y	Y	-42.3812	172.4031	Rolleston Track
GW874	Y	Y	South	F	Y	Y	Y	-42.3812	172.4031	Rolleston Track
GW875	Y	Y	South	F	Y	Y	Y	-42.3812	172.4031	Rolleston Track
GW877	x	Y	South	F	Y	Y	Y	-42.3812	172.4031	Rolleston Track
GW878	Y	Y	South	F	x	x	x	-42.3812	172.4031	Rolleston Track
GW462a	Y	Y	South	F	Y	Y	Y	-42.3141	172.1177	Lewis Pass
GW463a	Y	Y	South	F	Y	x	Y	-42.3141	172.1177	Lewis Pass
GW462b	x	Y	South	F	x	x	x	-42.3141	172.1177	Lewis Pass
GW1017	Y	Y	South	F	Y	Y	Y	-42.3141	172.1177	Lewis Pass
GW1018	Y	Y	South	F	x	Y	Y	-42.3141	172.1177	Lewis Pass
GW1019	Y	Y	South	F	Y	Y	Y	-42.3332	172.1674	Lewis Pass
GW1020	Y	Y	South	F	Y	Y	Y	-42.3332	172.1674	Lewis Pass
GW1021	Y	Y	South	F	Y	Y	Y	-42.3240	172.1283	Lewis Pass
GW1022	Y	Y	South	F	x	Y	Y	-42.3141	172.1177	Lewis Pass

GW1025	Y	Y	South	F	Y	Y	Y	-42.3141	172.1177	Lewis Pass
GW147	Y	Y	South	F	x	x	x	-42.0357	171.3885	Paparoa NP
GW758	Y	Y	South	F	x	x	x	-41.7660	171.7737	Denniston
GW864	Y	Y	South	F	Y	Y	Y	-41.7580	171.8055	Denniston
GW133	Y	Y	South	M	x	x	x	-43.1383	171.7401	Craigieburn
GW909	Y	Y	South	M	x	x	x	-42.9462	171.5644	Arthur's Pass
GW910A	Y	Y	South	M	x	x	x	-42.9462	171.5644	Arthur's Pass
GW915B	Y	Y	South	M	x	x	x	-42.9462	171.5644	Arthur's Pass
GW922A	Y	Y	South	M	x	x	x	-42.4693	171.2526	Greymouth
GW1011	Y	Y	South	M	Y	Y	Y	-42.3141	172.1177	Lewis Pass
GW202	Y	Y	South	x	x	x	x	-45.5927	167.9514	Takitimu Range
GW259	x	Y	South	x	x	Y	Y	-43.9073	168.9084	Haast
GW261	Y	Y	South	x	x	Y	Y	-43.9073	168.9084	Haast
GW150	Y	Y	South	x	x	x	x	-43.3879	170.1856	Franz Josef
GW130	Y	Y	South	x	x	x	x	-43.1383	171.7401	Craigieburn
GW909C	Y	Y	South	x	x	x	x	-42.9462	171.5644	Arthur's Pass
GW909A	Y	Y	South	x	x	x	x	-42.9462	171.5644	Arthur's Pass
GW911A	Y	Y	South	x	x	x	x	-42.9462	171.5644	Arthur's Pass
GW911B	Y	Y	South	x	x	x	x	-42.9462	171.5644	Arthur's Pass
GW913B	Y	Y	South	x	x	x	x	-42.9462	171.5644	Arthur's Pass
GW913C	Y	Y	South	x	x	x	x	-42.9462	171.5644	Arthur's Pass
GW914B	Y	Y	South	x	x	x	x	-42.9462	171.5644	Arthur's Pass
GW914C	Y	Y	South	x	x	x	x	-42.9462	171.5644	Arthur's Pass
GW145	Y	Y	South	x	x	x	x	-42.8016	171.5705	Arthur's Pass
GW922D	Y	Y	South	x	x	x	x	-42.4693	171.2526	Greymouth
GW922E	Y	Y	South	x	x	x	x	-42.4693	171.2526	Greymouth
GW924B	Y	Y	South	x	x	x	x	-42.4693	171.2526	Greymouth
GW925B	Y	Y	South	x	x	x	x	-42.4693	171.2526	Greymouth
GW152	Y	Y	South	x	x	x	x	-42.0372	171.3879	Paparoa NP
GW484	x	Y	South	x	x	x	x	-41.8017	172.8502	St Arnaud
GW214	Y	Y	South	x	x	x	x	-41.5506	172.5332	Mt Owen
GW694	Y	Y	South	x	x	x	x	-41.1902	172.7473	Pelorus Bridge
GW217	Y	Y	South	x	x	x	x	-41.1536	172.3749	Kahurangi NP
GW829	x	Y	South	x	x	x	x	-42.6479	171.0626	Awatuna
GW27	Y	NA	NA	NA	NA	NA	NA	-40.6747	172.4804	Paturau River

Appendix 3.3. Male and female PCA loadings.

Male PCA loadings			Female PCA loadings		
	PC1	PC2		PC1	PC2
PL	0.41	-0.01	PL	0.37	-0.19
FL	0.40	0.07	FL	0.39	0.17
BL	0.33	-0.15	BL	0.36	0.09
FW	0.37	0.13	FW	0.34	-0.28
HL	0.33	-0.37	HL	0.36	0.20
HW	0.35	-0.31	HW	0.39	0.04
PW	0.39	0.19	PW	0.37	-0.26
SG (relative)	0.03	0.63	OV (relative)	-0.05	0.65
SG	0.22	0.54	OV	0.19	0.56

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Chapter 4. Descriptions of five ground wētā species

Introduction

Anostostomatidae of New Zealand

Wētā (Insecta: Orthoptera: Anostostomatidae) are an important and prominent invertebrate group in New Zealand forests (Brockie 1992). The family in New Zealand comprises three main lineages, tree (*Hemideina* White, 1846) and giant (*Deinacrida* White, 1842) wētā, tusked wētā (*Anisoura* Ander, 1938, *Motuweta* Johns, 1997) and ground wētā (*Hemiandrus* Ander, 1938) (Johns 1997; Trewick and Morgan-Richards 2004; Trewick and Morgan-Richards 2005). Together, they comprise some 60 species that occupy diverse habitats from lowland forest to the alpine zone. Among them, the ground wētā are the most speciose but also the most poorly characterized and in need of most taxonomic and ecological work. Johns (1997) estimated that there might be as many as 42 undescribed species (pers comm) but there are eleven described to date (Jewell 2007; Johns 1997; Johns 2001; Taylor Smith *et al.* 2013 (see chapter 6)). In addition an estimated ten undescribed species of *Hemiandrus* are endemic to Australia (Johns 1997), although molecular data indicate that these may not be monophyletic with New Zealand *Hemiandrus* (Pratt *et al.* 2008).

Hemiandrus maculifrons species complex

Ground wētā (*Hemiandrus*) are found throughout North and South Islands, as well as on numerous offshore islands. Most species of ground wētā have restricted ranges, but *H. maculifrons* (Walker 1869) has a range that extends across both main islands. *Hemiandrus maculifrons* has been considered to be the most widespread ground wētā species, and also the most widespread New Zealand anostostomatid. In chapter 3 I showed that the unusually large range of *H. maculifrons* comprises a cryptic species complex. The concordance of morphological and genetic data indicated that the complex consists of three species each of which is fairly widespread with all three lineages present in northern South Island, an area of high diversity and endemism (Fig. 4.1). Within this region, northwest Nelson has the highest endemism (chapter 1), and here a fourth distinct *Hemiandrus maculifrons* complex mtDNA lineage was found (chapter 3).

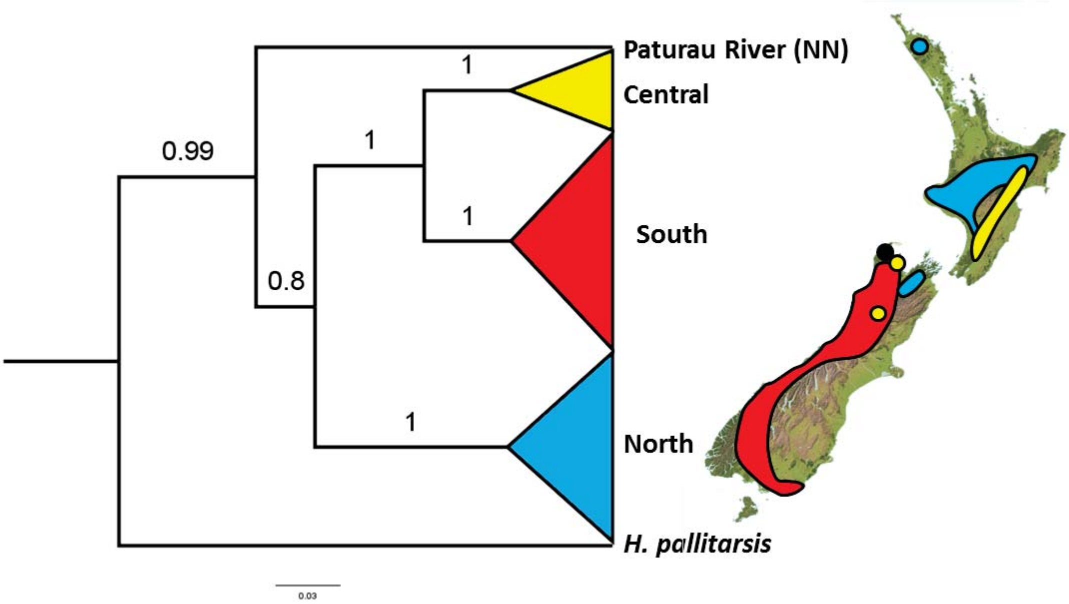

Figure 4.1. Bayesian mtDNA COI phylogeny from chapter 1 showing the relationships among species of the *Hemiandrus maculifrons* cryptic species complex, and their minimum distribution in New Zealand.

Who is *Hemiandrus maculifrons*?

Hemiandrus maculifrons was originally described by Walker (1869). Johns (1997) synonymised two described *Hemiandrus* species, *H. maori* (Pictet and Saussure 1893) and *H. gracilis* (Salmon 1950), with *H. maculifrons*. The finding that *H. maculifrons* consists of multiple species requires the revision of these names.

The holotype for *H. maculifrons* (*Libanasa (?) maculifrons*) (Walker 1869), is a damaged female from an unknown New Zealand location, with a description that could be applied to multiple ground wētā. However, in his species description, Walker (1869) gave the length of this specimen as 7.5 lines (approximately 15.9mm). Only some populations of the southern-most species of this complex have such a large body size (Fig. 4.2) indicating that the species that he described was the southern-most species.

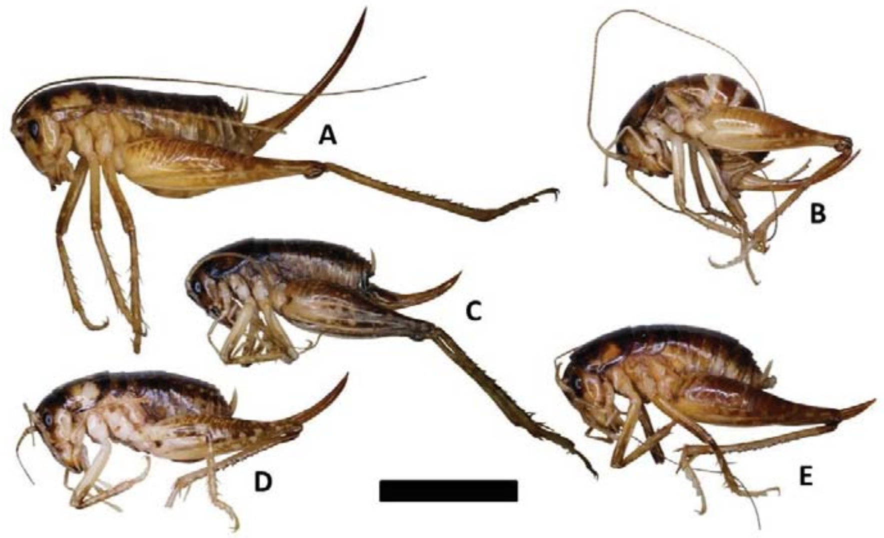

Figure 4.2. Representative female individuals of each of the five ground wētā species described here. **A.** *Hemiandrus maculifrons*. **B.** *Hemiandrus* PATURAU[2]. **C.** *Hemiandrus* ALIUS. **D.** *Hemiandrus* CENTRAL. **E.** *Hemiandrus* NORTH. Scale bar represents 10mm.

Pictet and Saussure (1893) based their description on specimens found near Mt Cook (Hutton 1897), which falls within the range of the southern-most species. Furthermore they state that below the middle of the hind tibiae there is a small spine either side and that the ovipositor is the length of the femur. These features are consistent with the southern-most species but not the central or northern species. Salmon (1950) also based his description on species collected from Southland, Westland, Fiordland and Mt Arthur, which are all localities with populations of the southern species of *H. maculifrons*. Therefore, although *H. maculifrons* is a species complex, all three of these species descriptions appear to have described the same species of this complex. Because of this, the southern-most species of this complex will retain the name *H. maculifrons* while the others will be given new names.

Also described here is a species formerly known by the tag-name[3] *H.* "alius" (Johns 2001), which is closely related to *H. maculifrons* (Pratt *et al*. 2008, Fig 6). Like species within the *H. maculifrons* species complex, *H.* "alius" is also found in primary native forest and is sympatric with and morphologically similar to these species.

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[2] Note that the names of species described in this thesis are not species names but will act as placeholders until the descriptions are formalised. These placeholder names will be used to refer to these species from this point on in this thesis.

[3] A tag-name is an informal name that indicates an entity that may be a separate species, monophyletic group or separate interbreeding population of uncertain taxonomic rank (Leschen *et al*. 2009).

Materials and methods

Taxonomic methods

Specimens were examined from a variety of sources: Department of Conservation (DoC) pitfall traps (Sinclair and Stringer 2003), New Zealand Arthropod Collection (NZAC), Auckland Museum of New Zealand (AMNZ) and the Phoenix Lab insect collection at Massey University, Palmerston North. All specimens were dry or preserved in 70-95% ethanol. Specimens were examined using an Olympus SZX7 Zoom Stereomicroscope with an attached SC100 digital camera.

176 female wētā and 76 male wētā (total 252) were measured and examined for their morphology (Appendix 4.1). Body length (partial body length: length from frons to distal margin of metanotum), head width, head length, pronotum length, pronotum width, femur width, femur length, ovipositor length and subgenital plate length measurements were made using digital callipers. Spines of the tibiae were counted. The hind tibial spines were counted on each leg of each individual and then averaged for each of superior prolateral spines, superior retrolateral spines and inferior spines. The stridulatory pegs of the inner hind femur and the first three abdominal tergites on the right hand side of each wētā were counted. Photographs of male terminalia, female ovipositors, mid and hind tibiae and pronota were captured using the Olympus Image Analysis Software. Photographs of heads and whole specimens were captured using a Canon EOS 40D digital camera. Specimens from additional locations were examined to assess the distributions of each species (Appendix 4.2) and distributions were mapped with the software ArcMap from ArcGIS 10.1 (ESRI, Redlands, CA, USA).

Abbreviations

Prefixes: GW is used for ground wētā in the Phoenix Lab collection (Massey University, Palmerston North); DOCROD, DoC pitfall specimens; AMNZ, Auckland Museum specimens; NZAC, New Zealand Arthropod Collection specimens; FD is used for a single specimen collected by Eric Edwards (DoC) from Fiordland.

Anatomy: BL, partial body length; HW, head width; HL, head length; PL, pronotum length; PW pronotum width; FW, femur width; FL; femur length; OV, ovipositor length; SG, subgenital plate length; T1, first abdominal tergite; T2, second abdominal tergite; T3, third abdominal tergite; T7, seventh abdominal tergite; T8, eighth abdominal tergite; T9, ninth abdominal tergite; T10, tenth abdominal tergite; MP3, third segment of the maxillary palps; MP4, fourth segment of the maxillary palps; MP5, fifth segment of the maxillary palps.

Area codes (Crosby *et al.* 1976): SD, Marlborough Sounds; MB, Marlborough; NN, Nelson; BR, Buller; NC, North Canterbury; MC, Mid Canterbury; WD, Westland; FD, Fiordland; OL, Otago Lakes; CO, Central Otago; DN, Dunedin; SL, Southland; TK, Taranaki; BP, Bay of Plenty; GB, Gisborne; HB, Hawke's Bay; RI, Rangitikei; WN, Wellington; ND, Northland; AK, Auckland; WO, Waikato; CL, Coromandel; TO, Taupo; WI, Wanganui.

Hemiandrus species descriptions

Class: Insecta
Order: Orthoptera
Suborder: Ensifera
Superfamily: Tettigoniodea
Family: Anostostomatidae Saussure (1859)
Genus: *Hemiandrus* Ander (1938)
Type species: *Hemiandrus furcifer* Ander (1938)

Re-description of *Hemiandrus maculifrons*

Diagnosis: A small to medium-sized ground wētā found in forests of the South Island, New Zealand, with the following traits: head and body mostly brown with some pale areas including large lateral and dorsal pale patches on the pronotum; the three apical segments of the maxillary palps with fine microsetae; mid tibiae with four spines along the inferior retrolateral angle (excluding apical spine); hind tibiae with two to four inferior articulated spines (rarely one); male subgenital plate short, with a flat, slightly U-shaped or notched apical margin; adult male cerci blunt; male T9 bilobed with acute lobes; females with a very long, gently curved ovipositor.

Description:

Adult body size: see Fig. 4.2, Table 4.1.

Head. Head shiny, darker brown on top with faint pale dorsal midline; gena cream or mottled cream and brown; frons and clypeus mottled, rugose; labrum cream and/or brown, setose; mandibles cream and brown, dark distally; scape, pedicel and antennomeres cream and light brown; antennae longer than body; flagella proximally smooth (12-14 antennomeres), distal antennomeres covered by short fine microsetae; eyes black; vertex with raised subtriangular fastigium; ocellar spots white; maxillary palps cream with bulbous apices and with widely spaced setae and covered by short, fine microsetae (MP5 100% pilose, MP4 100%, MP3 ~50%) (Fig. 4.3).

Table 4.1. Body measurements (in mm), spine numbers and peg numbers for both sexes of five species. For measurements the mean and standard error are reported. For relative sizes and counts the mean and range are reported.

Species:	***maculifrons***	**CENTRAL**	**NORTH**	**ALIUS**	**PATURAU**
Males	n=15	n=9	n=33	n=16	n=1
PL	4.17 (3.82-4.76)	4.22 (3.80-4.55)	4.07 (3.56-4.74)	4.49 (4.23-4.73)	3.84
PW	4.12 (3.70-4.73)	4.14 (3.61-4.53)	4.17 (3.73-4.89)	4.27 (4.13-4.42)	4.27
FL	11.86 (10.53-13.93)	10.29 (9.67-10.91)	10.70 (9.54-13.12)	10.28 (9.54-11.46)	10.96
FW	3.21 (2.80-3.66)	3.24 (2.85-3.44)	3.23 (2.74-3.86)	3.42 (3.12-3.70)	3.57
HL	5.44 (4.84-5.99)	5.39 (5.16-5.73)	4.87 (4.22-5.61)	4.95 (4.57-5.25)	5.00
HW	3.75 (3.43-4.26)	3.60 (3.41-3.74)	3.30 (3.04-3.85)	3.10 (2.84-3.38)	3.24
BL (partial)	8.00 (7.20-8.94)	7.38 (6.76-7.82)	7.32 (6.13-9.18)	7.29 (6.96-7.42)	6.07
SG	2.23 (1.91-2.61)	2.13 (1.80-2.35)	2.82 (2.38-3.50)	2.01 (1.71-2.24)	1.93
Relative SG (SG/PL)	0.54 (0.43-0.65)	0.51 (0.42-0.55)	0.69 (0.66-0.80)	0.44 (0.39-0.47)	0.50
Relative FL (FL/HW)	3.16 (2.98-3.54)	2.86 (2.77-2.95)	3.24 (2.95-3.5)	3.23 (3.12-3.38)	3.38
Females	n=63	n=24	n=69	n=26	n=1
PL	4.58 (3.40-5.54)	4.60 (4.04-5.34)	4.76 (3.98-6.05)	4.76 (4.25-5.43)	4.31
PW	4.61 (3.53-5.55)	4.54 (4.15-5.17)	4.93 (4.15-5.91)	4.56 (3.99-5.24)	4.41
FL	12.79 (9.89-15.70)	11.48 (10.69-12.31)	12.03 (10.19-14.38)	11.16 (10.02-12.41)	11.69
FW	3.46 (2.72-4.13)	3.41 (3.04-3.97)	3.70 (3.00-4.48)	3.49 (3.10-4.08)	3.24
HL	6.11 (5.06-6.96)	6.01 (5.63-6.54)	5.77 (4.95-6.68)	5.54 (4.82-6.29)	5.79
HW	4.10 (3.36-4.88)	4.05 (3.86-4.43)	3.96 (3.42-4.69)	3.55 (3.00-3.97)	3.90
BL (partial)	8.91 (6.88-10.93)	8.42 (7.36-11.05)	8.54 (6.76-10.45)	7.95 (7.13-9.30)	7.93
OV	12.03 (9.58-15.10)	9.35 (8.52-10.80)	9.44 (7.79-11.55)	7.63 (6.81-8.55)	10.21
Relative OV (OV/FW)	3.47 (3.05-4.03)	2.69 (2.34-3.10)	2.53 (2.09-2.89)	2.19 (1.82-2.41)	3.15
Relative FL (FL/HW)	3.11 (2.80-3.47)	2.82 (2.66-3.02)	3.04 (2.81-3.23)	3.14 (2.98-3.45)	3.00
Males & females	n=71	n=33	n=102	n=26	n=2
Mean prolateral superior spines of hind tibiae	14 (11-18)	14 (11-16)	15 (10-20)	14 (12-17)	15 (14-15)
Mean retrolateral superior spines of hind tibiae	13 (10-17)	13 (11-15)	15 (10-18)	12 (10-16)	15 (14-15)
Mean inferior hind tibial spines	2 (2-4, rarely 1)	1 (rarely 2)	1 (1-2)	0	1
Hind femoral pegs	33 (0-66)	22 (10-55)	6 (0-96)	0	11 (7-15)
Pegs T1	31 (4-71)	32 (18-54)	45 (18-80)	21 (15-25)	22 (20-23)
Pegs T2	41 (22-66)	41 (26-68)	47 (23-75)	21 (16-25)	23 (15-31)
Pegs T3	37 (17-63)	38 (10-63)	39 (15-75)	21 (11-25)	23 (20-25)

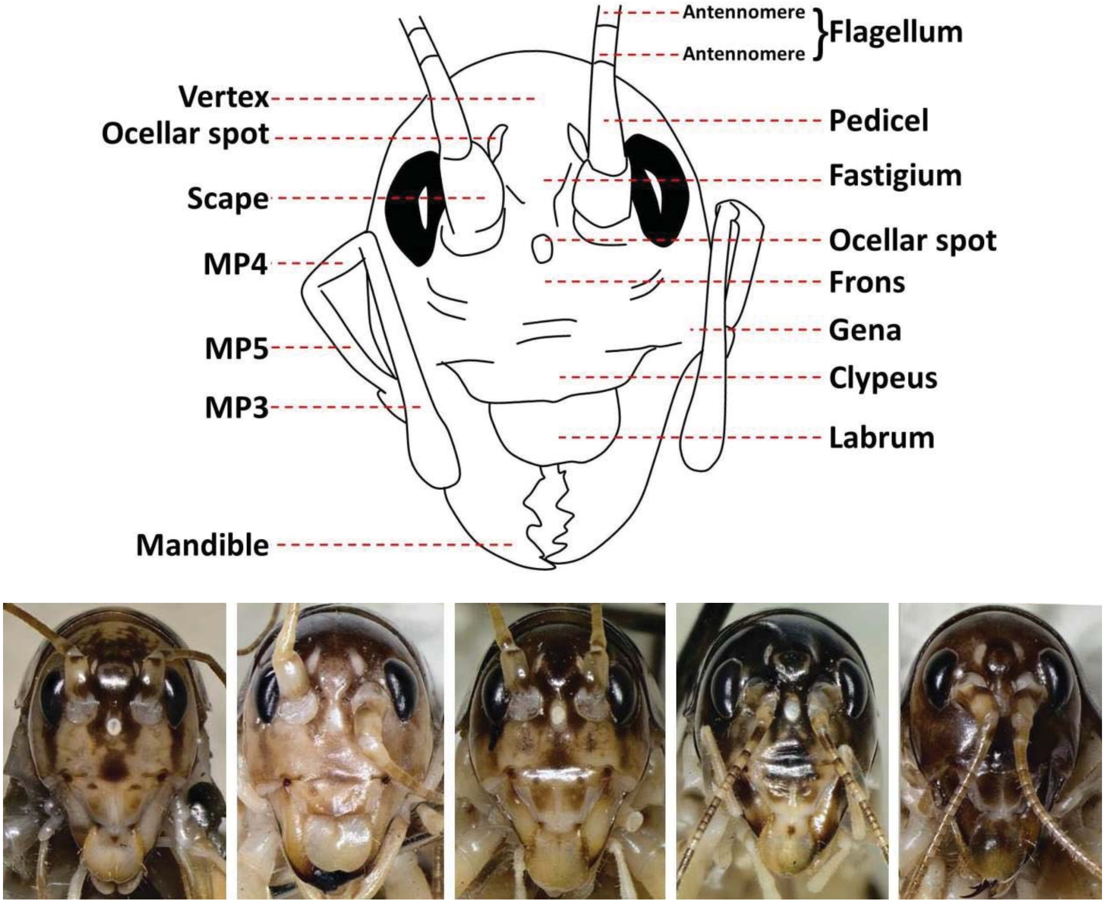

Figure 4.3. Top: diagram of *Hemiandrus* head showing the position of features mentioned in text. Bottom, from left to right: *H. maculifrons*, *H.* PATURAU, *H.* NORTH, *H.* CENTRAL , *H.* ALIUS. *Hemiandrus* ALIUS is a very dark coloured species including dark gena, frons, clypeus, labrum and mandibles.

Thorax. Pronotum, mesonotum and metanotum brown with a faint pale dorsal mid-line which extends from the head to the abdominal tergites; pronotum shiny and smooth dorsally, rugose ventrally, approximately as long as wide, large lateral pale patches and the dorsal area mottled with large pale patches (Fig. 4.4). Thoracic sterna cream and/or light brown; each with two blunt spines which are long and thin on the prosternum and short and wide on the meso- and metasterna (see Fig. 5A, 5B (ventral aspects) in Taylor Smith *et al.* (2013) (chapter 6).

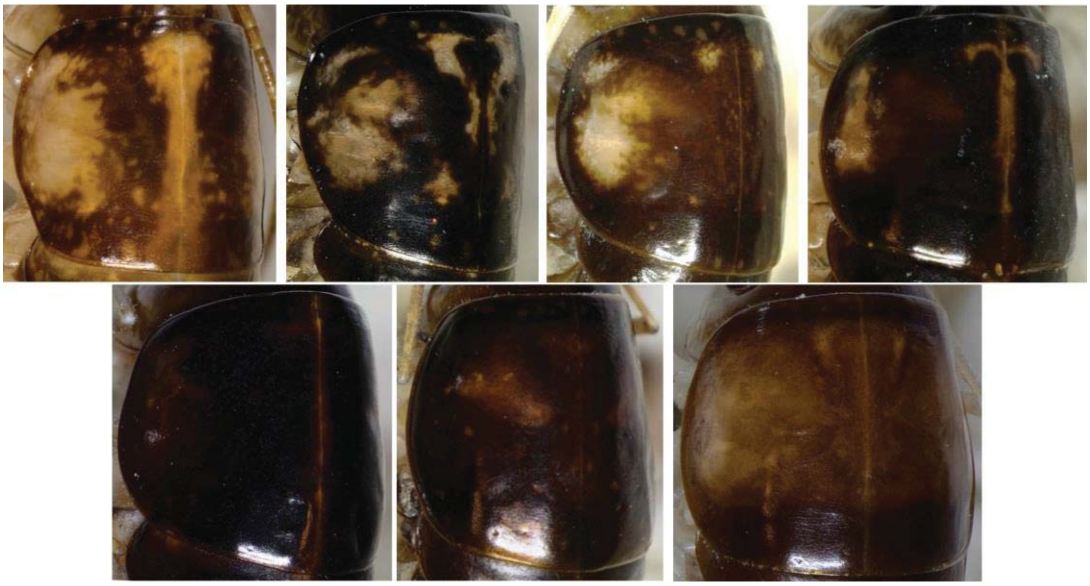

Figure 4.4. Patterning of the pronotum varies both within and between species. Top row: *H. maculifrons*, *H.* CENTRAL (South Island), *H.* CENTRAL (North Island), *H.* NORTH. Bottom row: *H.* NORTH, *H.* ALIUS, *H.* PATURAU.

Legs. Long (hind femora 2.8 to 3.5 times head width); coxae and trochanters cream; fore and mid coxae with spinous lobes; femora cream at base becoming brown with cream spots; tibiae brown with cream spots; fore tibiae lacking tympanum. Fore tibiae with 13 cream articulated spines with brown tips arranged as follows: four apicals; one positioned medially on superior prolateral angle; none on superior retrolateral angle; four along the inferior prolateral angle; four along the inferior retrolateral angle. Mid tibiae with 17 cream articulated spines with brown tips arranged as follows: four apicals; two along the superior prolateral angle; three along the superior retrolateral angle; four along the inferior prolateral angle; four along the inferior retrolateral angle (Fig. 4.5). Hind tibiae with 30-47 spines arranged as follows: two pairs of short apical spines, one pair of long apical spines, one pair of long subapical spines; 11-18 fixed spines along the superior prolateral angle; 10-17 fixed spines along the superior retrolateral angle; two to four (usually two, rarely one) small articulated spines on the ventral side of the tibiae (Fig. 4.6). Tarsi cream, setose, four-segmented, first segment with foot-pad divided into two, other segments with a single foot-pad. The first segment sparsely setose, segments two and three range from bare to pilose, 4th is pilose with some setae. The inner side of the hind femora with 0-66 small pegs.

Abdomen. Abdominal tergites brown with some pale spots, paler laterally; T1 with 4-71 stridulatory pegs; T2 with 22-66 stridulatory pegs; T3 with 17-63 stridulatory pegs; pleural membrane light brown or cream with stridulatory pegs; sternites cream.

Figure 4.5. Inferior mid tibial spines. From left to right: *H.* ALIUS, *H.* NORTH, *H.* PATURAU, *H. maculifrons*, *H.* CENTRAL. Spines are positioned in pairs with a single apical pair and four pairs spaced along the inferior mid tibiae. *Hemiandrus* ALIUS lacks a proximal spine along the retrolateral angle leaving a single unpaired spine (arrow).

Figure 4.6. Inferior hind tibial spines of species described here. From left to right: *H.* ALIUS, *H.* CENTRAL, *H. maculifrons* *H.* PATURAU, *H.* NORTH. *Hemiandrus maculifrons* usually has two spines, but sometimes more and in a rare case, only a single spine. *Hemiandrus* ALIUS has no articulated spines on the inferior side of the hind tibiae. *Hemiandrus* CENTRAL and *H.* PATURAU usually have only a single spine, while *H.* NORTH usually has one or two spines.

Males. ninth abdominal tergite (T9) paler than other tergites, apical margin with two acute lobes (Fig. 4.7); T10 with two dark hooks usually positioned between the lobes of T9 but sometimes hidden beneath; subgenital plate short (mean 2.23mm, 43-65% of pronotum length) with a flat, slightly U-shaped or notched distal margin (Fig. 4.8); styles short; conical paraprocts; cerci blunt, cream, setose.

Females. T7 simple or with a small median lobe; T8 simple or with a small median lobe; T9 bilobed or slightly notched; cerci cream, long, pointed, with long setae; ovipositor gently curved, very long (mean 12.03 mm), 3.1-4.0 times hind femur width; subgenital plate triangular; seventh abdominal sternite with paired pits situated anteriorly either side of the mid line.

Distribution: NN, BR, NC, MC, WD, FD, OL, CO, DN, SL (See Fig. 4.9A, Appendix 4.1, 4.2).

Figure 4.7. Male ninth abdominal tergites. Also visible are the falci, cerci and paraprocts. Top row: *H. maculifrons*, *H.* CENTRAL, *H.* NORTH. Bottom row: *H.* ALIUS, *H.* PATURAU.

Figure 4.8. Male subgenital plates. Top row: *H. maculifrons*, *H.* CENTRAL, *H.* NORTH. Bottom row: *H.* NORTH, *H.* ALIUS, *H.* PATURAU.

Species description: *Hemiandrus* CENTRAL

Diagnosis: A small ground wētā found in forests of North Island and northern South Island, New Zealand, with the following traits: head and body mostly brown with some pale areas including large lateral and dorsal pale patches on the pronotum; the three apical segments of the maxillary palps with fine microsetae; mid tibiae with four spines along the inferior retrolateral angle (excluding apical spine); hind tibiae with a single inferior articulated spine (rarely two); male subgenital plate short, with a flat, slightly U-shaped or notched apical margin; adult male cerci blunt; male T9 bilobed with acute lobes; females with a long, gently curved ovipositor

Description:

Adult body size: see Fig. 4.2, Table 4.1.

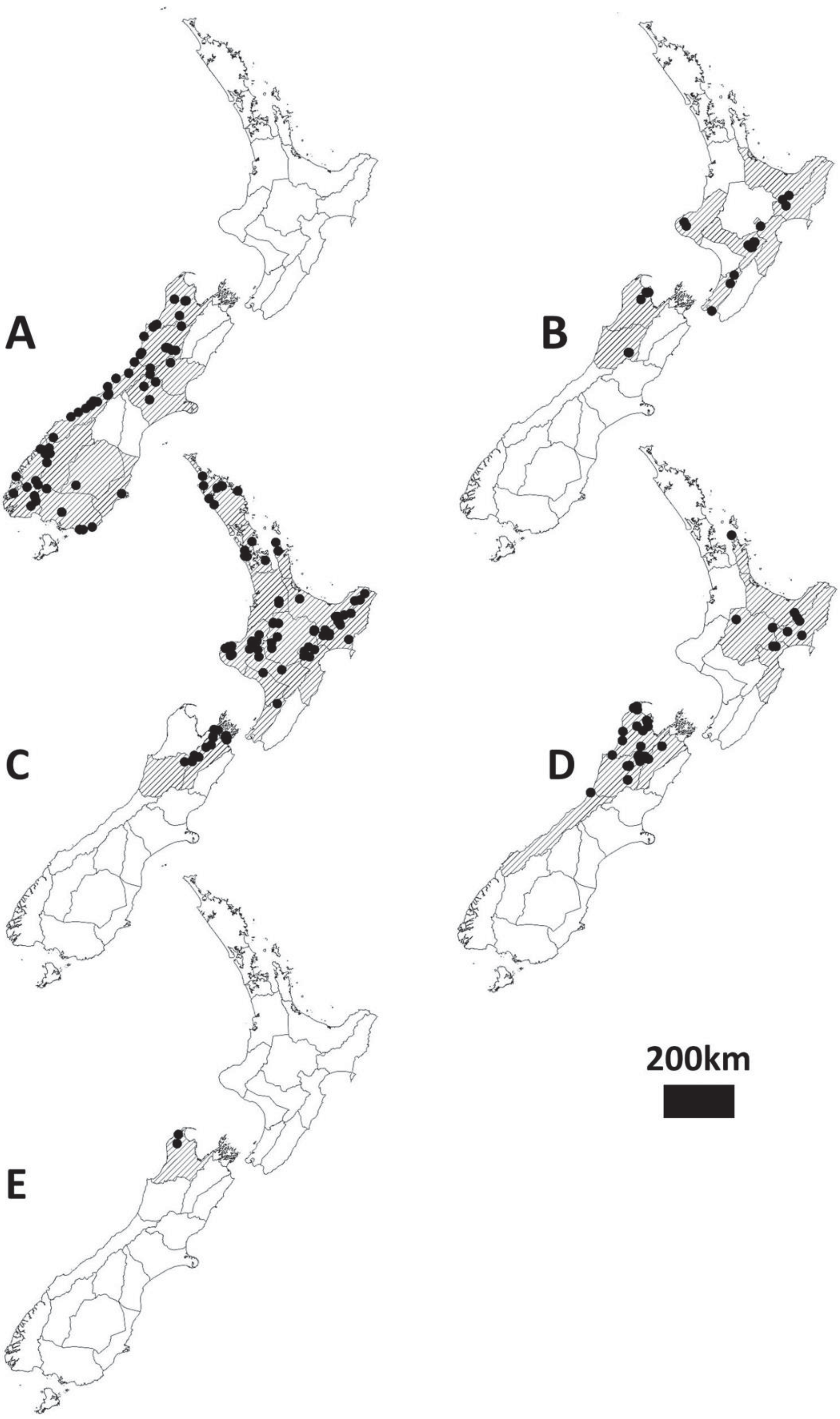

Figure 4.9. Species' distributions and the Crosby regions (Crosby *et al.* 1976) in which each species is found (hatched areas). **A.** *Hemiandrus maculifrons.* **B.** *Hemiandrus* CENTRAL **C.** *Hemiandrus* NORTH **D**. *Hemiandrus* ALIUS **E.** *Hemiandrus* PATURAU.

Head. Head shiny, darker brown on top with faint pale dorsal midline; gena cream or mottled cream and brown; frons and clypeus mottled, rugose; labrum cream and/or brown, setose; mandibles cream and brown, dark distally; scape, pedicel and antennomeres cream and light brown; antennae longer than body; flagella proximally smooth (9- 12 antennomeres), distal antennomeres covered by short fine microsetae; eyes black; vertex with raised subtriangular fastigium; ocellar spots white; maxillary palps cream with bulbous apices and with widely spaced setae and covered by short, fine microsetae (MP5 100% pilose, MP4 100%, MP3 ~50%) (Fig. 4.3).

Thorax. Pronotum, mesonotum and metanotum brown with a faint pale dorsal mid-line which extends from the head to the abdominal tergites; pronotum shiny and smooth dorsally, rugose ventrally, approximately as long as wide, large lateral pale patches and the dorsal area with little to extensive mottling and pale patches (Fig. 4.4). Thoracic sterna cream and/or light brown; each with two blunt spines which are long and thin on the prosternum and short and wide on the meso- and metasterna.

Legs. Long (hind femora 2.7 to 3.0 times head width); coxae and trochanters cream; fore and mid coxae with spinous lobes; femora cream at base becoming brown with cream spots; tibiae brown with cream spots; fore tibiae lacking tympanum. Fore tibiae with 13 cream articulated spines with brown tips arranged as follows: four apicals; one positioned medially on superior prolateral angle; none on superior retrolateral angle; four along the inferior prolateral angle; four along the inferior retrolateral angle. Mid tibiae with 17 cream articulated spines with brown tips arranged as follows: four apicals; two along the superior prolateral angle; three along the superior retrolateral angle; four along the inferior prolateral angle; four along the inferior retrolateral angle (Fig. 4.5). Hind tibiae with 31-41 spines arranged as follows: two pairs of short apical spines, one pair of long apical spines, one pair of long subapical spines; 11-16 fixed spines along the superior prolateral angle; 11-15 fixed spines along the superior retrolateral angle; one (rarely two) small articulated spine on the ventral side of the tibiae (Fig. 4.6). Tarsi cream, setose, four-segmented, first segment with foot-pad divided into two, other segments with a single foot-pad. The first segment sparsely setose, segments two and three range from bare to pilose, 4th is pilose with some setae. The inner side of the hind femora with 10-55 small pegs.

Abdomen. Abdominal tergites brown with some pale spots, paler laterally; T1 with 18-54 stridulatory pegs; T2 with 26-68 stridulatory pegs; T3 with 10-63 stridulatory pegs; pleural membrane light brown or cream with stridulatory pegs; sternites cream and/or light brown.

Males. Ninth abdominal tergite (T9) paler than other tergites, apical margin with two acute lobes (Fig. 4.7); T10 with two dark hooks usually positioned between the lobes of T9 but sometimes hidden beneath; subgenital plate short (mean 2.13mm, 42-55% of pronotum length) with a flat, slightly U-shaped or notched distal margin (Fig. 4.8); styles short; conical paraprocts; cerci blunt, cream, setose.

Females. T7 simple or with a small median lobe; T8 simple or with a small median lobe; T9 bilobed or slightly notched; cerci cream, long, pointed, with long setae; ovipositor gently curved, long (mean 9.35 mm), 2.3-3.1 times hind femur width; subgenital plate triangular; seventh abdominal sternite with paired pits situated anteriorly either side of the mid line.

Type specimens: TBA

Distribution: TK, BP, GB, HB, RI, WN, NN, BR (See Fig. 4.9B, Appendix 4.1, 4.2).

Remarks: This species is similar to *H.* PATURAU, but *H.* CENTRAL usually has a shorter ovipositor (relatively). Furthermore, on South Island, *H.* CENTRAL has extensive mottling of the pronotum, whereas on North Island, its pronotum pattern is more similar to that of *H.* PATURAU (Fig. 4.4).

Species description: *Hemiandrus* NORTH

Diagnosis: A small ground wētā found throughout forests of North Island and northern South Island, New Zealand, with the following traits: head and body mostly brown with some pale areas sometimes including small lateral and sometimes small dorsal pale patches on the pronotum; the three apical segments of the maxillary palps with fine microsetae; the mid tibiae with four spines along the inferior retrolateral angle (excluding apical spine); hind tibiae with a single inferior articulated spine (rarely two); male subgenital plate long, with deep V- or U-shape apical margin; Adult male cerci blunt; male T9 bilobed with obtuse, curved lobes; females with a long, gently curved ovipositor

Description:
Adult body size: see Fig. 4.2, Table 4.1.

Head. Head shiny, darker brown on top with faint pale dorsal midline; gena cream or mottled cream and brown; frons and clypeus mottled, rugose; labrum cream and/or brown, setose; mandibles cream and brown, dark distally; scape, pedicel and antennomeres cream and light brown; antennae longer than body; flagella proximally smooth (11-13 antennomeres), distal antennomeres covered by short fine microsetae; eyes black; vertex with raised subtriangular fastigium; ocellar spots white; maxillary palps cream with bulbous apices and with widely spaced setae and covered by short, fine microsetae (MP5 100% pilose, MP4 100%, MP3 ~50%) (Fig. 4.3).

Thorax. Pronotum, mesonotum and metanotum brown with a faint pale dorsal mid-line which extends from the head to the abdominal tergites; pronotum shiny and smooth dorsally, rugose ventrally, approximately as long as wide, with or without small lateral pale patches and the dorsal area with or without pale patterning around midline and anterior edge (Fig. 4.4). Thoracic sterna cream and/or light brown; each with two blunt spines which are long and thin on the prosternum and short and wide on the meso- and metasterna.

Legs. Long (hind femora 2.8 to 3.5 times head width); coxae and trochanters cream; fore and mid coxae with spinous lobes; femora cream at base becoming brown with cream spots; tibiae brown with cream spots; fore tibiae lacking tympanum. Fore tibiae with 13 cream articulated spines with brown tips arranged as follows: four apicals; one positioned medially on superior prolateral angle; none on superior retrolateral angle; four along the inferior prolateral angle; four along the inferior retrolateral angle. Mid tibiae with 17 cream articulated spines with brown tips arranged as follows: four apicals; two along the superior prolateral angle; three along the superior retrolateral angle; four along the inferior prolateral angle; four along the inferior retrolateral angle (Fig. 4.5). Hind tibiae with 29-48 spines arranged as follows: two pairs of short apical spines, one pair of long apical spines, one pair of long subapical spines. 10-20 fixed spines along the superior prolateral angle; 10-18 fixed spines along the superior retrolateral angle; usually one or two small articulated spines on the ventral side of the tibiae (Fig. 4.6). Tarsi cream, setose, four-segmented, first segment with foot-pad divided into two, other segments with a single foot-pad. The first segment sparsely setose, segments two and three range from bare to pilose, 4th is pilose with some setae. The inner side of the hind femora with 0-96 small pegs.

Abdomen. Abdominal tergites brown, paler laterally; T1 with 18-80 stridulatory pegs; T2 with 23-75 stridulatory pegs ; T3 with 15-75 stridulatory pegs; pleural membrane light brown or cream with stridulatory pegs; sternites cream.

Males. Ninth abdominal tergite (T9) paler than other tergites, apical margin with two widely-spaced, obtuse lobes (Fig. 4.7); T10 with two dark hooks usually positioned beneath T9 but sometimes exposed; subgenital plate long (mean 2.82mm, 66-80% of pronotum length) with a deep V- to U-shaped distal margin (Fig. 4.8); styles short; paraprocts wide and irregularly shaped; cerci blunt, cream, setose.

Females. T7 simple or with a small median lobe; T8 simple or with a small median lobe; T9 bilobed or slightly notched; cerci cream, long, pointed, with long setae; ovipositor gently curved, long (mean 9.44 mm), 2.1-2.9 times hind femur width; subgenital plate triangular; seventh abdominal sternite with paired pits situated anteriorly either side of the mid line.

Type specimens: TBA

Distribution: ND, AK, WO, CL, BP, GB, TO, TK, RI, WI, WN, HB, SD, MB, BR (See Fig. 4.9C, Appendix 4.1, 4.2).

Remarks: *Hemiandrus* NORTH has a high level of morphological variation (chapter 3). Specimens from East Cape and Northland appear to be slightly larger with no or little patterning on their pronota. Males within these populations appear to have variable terminalia with the distal margins of their subgenital plates ranging from V-shaped to U-shaped. *Hemiandrus* NORTH specimens examined from all other locations had more extensive patterning on their pronota and males had U-shaped subgenital plates.

Species description: *Hemiandrus* ALIUS

Diagnosis: A small ground wētā found in forests of North and South Islands, New Zealand, with the following traits: head and body dark brown with cream and brown clypeus; sometimes with small pale patches on the pronotum; the three apical segments of the maxillary palps with fine microsetae; mid tibiae with three spines along the inferior retrolateral angle (excluding apical spine); hind tibiae with no inferior articulated spines; male subgenital plate short, narrower towards apex, with a flat or slightly U-shaped apical margin that may extend beyond the styles; adult male cerci pointed; male T9 bilobed with each lobe slightly pointed; females with a medium-length, strongly curved ovipositor with dark patches at its base.

Description:

Adult body size: see Fig. 4.2, Table 4.1.

Head. Shiny, dark with faint pale dorsal midline; gena sometimes slightly paler brown; frons and clypeus rugose, brown but clypeus with white mottling; labrum brown, sometimes also cream, setose; mandibles brown, darker distally; scape, pedicel and antennomeres light brown; antennae longer than body; flagella proximally smooth (six to ten antennomeres), distal antennomeres covered by short fine microsetae; eyes black; vertex with raised subtriangular fastigium; ocellar spots white; maxillary palps cream with bulbous apices and with widely spaced setae and covered by short, fine microsetae (MP5 100% pilose, MP4 100%, MP3 ~50%)(Fig. 4.3).

Thorax. Pronotum, mesonotum and metanotum dark brown with a faint pale dorsal mid-line which extends from the head to the abdominal tergites; pronotum shiny and smooth dorsally, rugose ventrally, approximately as long as wide, usually paler laterally and the dorsal area sometimes with small pale spots (Fig. 4.4). Thoracic sterna cream and/or light brown; each with two blunt spines which are long and thin on the prosternum and short and wide on the meso- and metasterna.

Legs. Long (hind femora 3.0 to 3.5 times head width); coxae and trochanters cream; fore and mid coxae with spinous lobes; femora cream at base becoming brown with cream spots; tibiae brown with cream spots; fore tibiae lacking tympanum. Fore tibiae with 13 cream articulated spines with brown tips arranged as follows: four apicals; one positioned medially on superior prolateral angle; none on superior retrolateral angle; four along the inferior prolateral angle; four along the inferior retrolateral angle. Mid tibiae with 16 cream articulated spines with brown tips arranged as follows: four apicals; two along the superior prolateral angle; three along the superior retrolateral angle; four along the inferior prolateral angle; three along the inferior retrolateral angle (Fig. 4.5). Hind tibiae with 30-41 spines arranged as follows: two pairs of short apical spines, one pair of long apical spines, one pair of long subapical spines; 12-17 fixed spines along the superior prolateral angle; 10-16 fixed spines along the superior retrolateral angle; no small articulated spine on the ventral side of the tibiae (Fig. 4.6). Tarsi cream, setose, four-segmented, first segment with foot-pad divided into two, other segments with a single foot-pad. The first segment sparsely setose, segments two and three range from bare to pilose, 4th is pilose with some setae. The inner side of the hind femora with no small pegs.

Abdomen. Abdominal tergites dark brown with some pale spots, paler laterally; T1 with 15-25 stridulatory pegs; T2 with 16-25 stridulatory pegs; T3 with 11-25 stridulatory pegs; pleural membrane dark brown with stridulatory pegs; sternites cream and/or light brown.

Males. Ninth abdominal tergite (T9) paler than other tergites, apical margin with two obtuse slightly pointed lobes (Fig. 4.7); T10 with two dark hooks positioned beneath T9; subgenital plate short (mean 1.78mm, 39-47% of pronotum length), narrower towards apex, with a flat or slightly U-shaped distal margin that may extend beyond the styles (Fig. 4.8); styles short; blunt cylindrical paraprocts; cerci cream, sharp, setose.

Females. T7 simple or with a small median lobe; T8 with a small median lobe; T9 slightly notched; cerci cream, long, pointed, with long setae; ovipositor very curved, dark patches at base (Fig. 4.10), medium length (mean 7.63 mm), 1.8-2.4 times hind femur width; subgenital plate triangular; seventh abdominal sternite with paired pits situated anteriorly either side of the mid line.

Figure 4.10. Top: *Hemiandrus* ALIUS has a shorter, darker, curvier ovipositor compared to other species described here. Bottom: *Hemiandrus maculifrons* ovipositor for comparison.

Type specimens: TBA

Distribution: CL, BP, GB, HB, TO, MB, NN, BR, WD (See Fig. 4.9D, Appendix 4.1, 4.2).

Remarks: This species is found in sympatry with the four other species described here and is most abundant in northwest Nelson. Johns (2001) reported this species to be found only in South Island, but it is also present in North Island.

Species description: *Hemiandrus* PATURAU

Diagnosis: A small ground wētā found in Kahurangi National Park, north west South Island, New Zealand, with the following traits: head and body mostly brown with some pale areas including small lateral and dorsal pale patches on the pronotum; the three apical segments of the maxillary palps with fine microsetae; the mid tibiae with four spines along the inferior retrolateral angle (excluding apical spine); hind tibiae with a single inferior articulated spine; male subgenital plate short, with a slightly notched apical margin; adult male cerci blunt, male T9 bilobed with acute lobes; females with a long, gently curved ovipositor.

Description:

Adult body size: see Fig. 4.2, Table 4.1.

Head. Head shiny, darker brown on top with faint pale dorsal midline; gena cream or mottled cream and brown; frons and clypeus mottled, rugose; labrum cream and/or brown, setose; mandibles cream and brown, dark distally; scape, pedicel and antennomeres cream and light brown; antennae longer than body; flagella proximally smooth (11-12 antennomeres), distal antennomeres covered by short fine microsetae; eyes black; vertex with raised subtriangular fastigium; ocellar spots white; maxillary palps cream with bulbous apices and with widely spaced setae and covered by short, fine microsetae (MP5 100% pilose, MP4 100%, MP3 ~50%) (Fig. 4.3).

Thorax. Pronotum, mesonotum and metanotum brown with a faint pale dorsal mid-line which extends from the head to the abdominal tergites; pronotum shiny and smooth dorsally, rugose ventrally, approximately as long as wide, small lateral pale patches and the dorsal area with pale patches either side of midline towards anterior edge (Fig. 4.4). Thoracic sterna cream and/or light brown; each with two blunt spines which are long and thin on the prosternum and short and wide on the meso- and metasterna.

Legs. Long (hind femora 3.0 to 3.4 times head width); coxae and trochanters cream; fore and mid coxae with spinous lobes; femora cream at base becoming brown with cream spots; tibiae brown with cream spots; fore tibiae lacking tympanum. Fore tibiae with 13 cream articulated spines with brown tips arranged as follows: four apicals; one positioned medially on superior prolateral angle; none on superior retrolateral angle; four along the inferior prolateral angle; four along the inferior retrolateral angle. Mid tibiae with 17 cream articulated spines with brown tips arranged as follows: four apicals; two along the superior prolateral angle; three along the superior retrolateral angle; four along the inferior prolateral angle; four along the inferior retrolateral angle (Fig. 4.5). Hind tibiae with 37-39 spines arranged as follows: two pairs of short apical spines, one pair of long apical spines, one pair of long subapical spines; 14-15 fixed spines along the superior prolateral angle; 14-15 fixed spines along the superior retrolateral angle; one small articulated spine on the ventral side of the tibiae (Fig. 4.6). Tarsi cream, setose, four-segmented, first segment with foot-pad divided into two, other segments with a single foot-pad. The first segment sparsely setose, segments two and three range from bare to pilose, 4th is pilose with some setae. The inner side of the hind femora with 7-15 small pegs.

Abdomen. Abdominal tergites brown, paler laterally; T1 with 20-23 stridulatory pegs; T2 with 15-31 stridulatory pegs; T3 with 20-25 stridulatory pegs; pleural membrane light brown with stridulatory pegs; sternites cream.

Males. Ninth abdominal tergite (T9) paler than other tergites, apical margin with two acutely-pointed lobes (Fig. 4.7); T10 with two dark hooks positioned beneath T9; subgenital plate short (1.93 mm, 50% of pronotum length) with a slightly notched distal margin (Fig. 4.8); styles short; conical paraprocts; cerci cream, blunt, setose.

Females. T7 simple; T8 simple; T9 slightly notched; cerci cream, long, pointed, with long setae; ovipositor gently curved, long (10.21 mm, 3.2 times hind femur width); subgenital plate triangular; seventh abdominal sternite with paired pits situated anteriorly either side of the mid line.

Type specimens: TBA

Distribution: Kahurangi National Park, NN (See Fig. 4.9E, Appendix 4.1, 4.2).

Remarks: This species appears to be highly range restricted; only being found on the north-western edge of northwest Nelson where it is sympatric with *H.* ALIUS. Its range restriction might be due to ecological competition, as two closely related species (*H. maculifrons* and *H.* CENTRAL) are also found in northwest Nelson. *Hemiandrus* PATURAU is similar to *H.* CENTRAL, but, unlike South Island *H.* CENTRAL that have extensive pronotal mottling, *H.* PATURAU has little mottling (Fig. 4.4).

Distinguishing between species

The five species described can be distinguished from one another by head and body colour, patterning of the pronotum, mid tibial and hind tibial spines, male terminalia size and shape, female and ovipositor length (Table 4.2)

The five species described here are easily distinguished from the majority of other ground wētā species by the combination of microsetae on the entire fourth segment of the maxillary palps and long ovipositors. They can further be distinguished by the absence of modifications of the female sixth abdominal sternite and presence of small pits on the seventh abdominal sternite. Note, however, that the seventh sternite pits are not always obvious due to being very shallow and sometimes hidden by the sixth sternite. Four other *Hemiandrus* species also share these traits (i.e. MP4 100% pilose, long ovipositor, no female sixth sternite modification): *H. subantarcticus* (Salmon 1950), *H. fiordensis* (Salmon 1950), *H. nitaweta*[4] (Jewell 2007) and *H.* "madisylvestris" (Johns 2001). *Hemiandrus subantarcticus* is found on Snares Island, while the latter three species are found on South Island. *Hemiandrus nitaweta* is strikingly coloured with red and black pronotal patterning (Jewell 2007) and eight or fewer fixed spines along each of the superior pro- and retrolateral angles of the hind tibiae (pers obs) whereas the species described here all have ten or more spines. *Hemiandrus fiordensis* can

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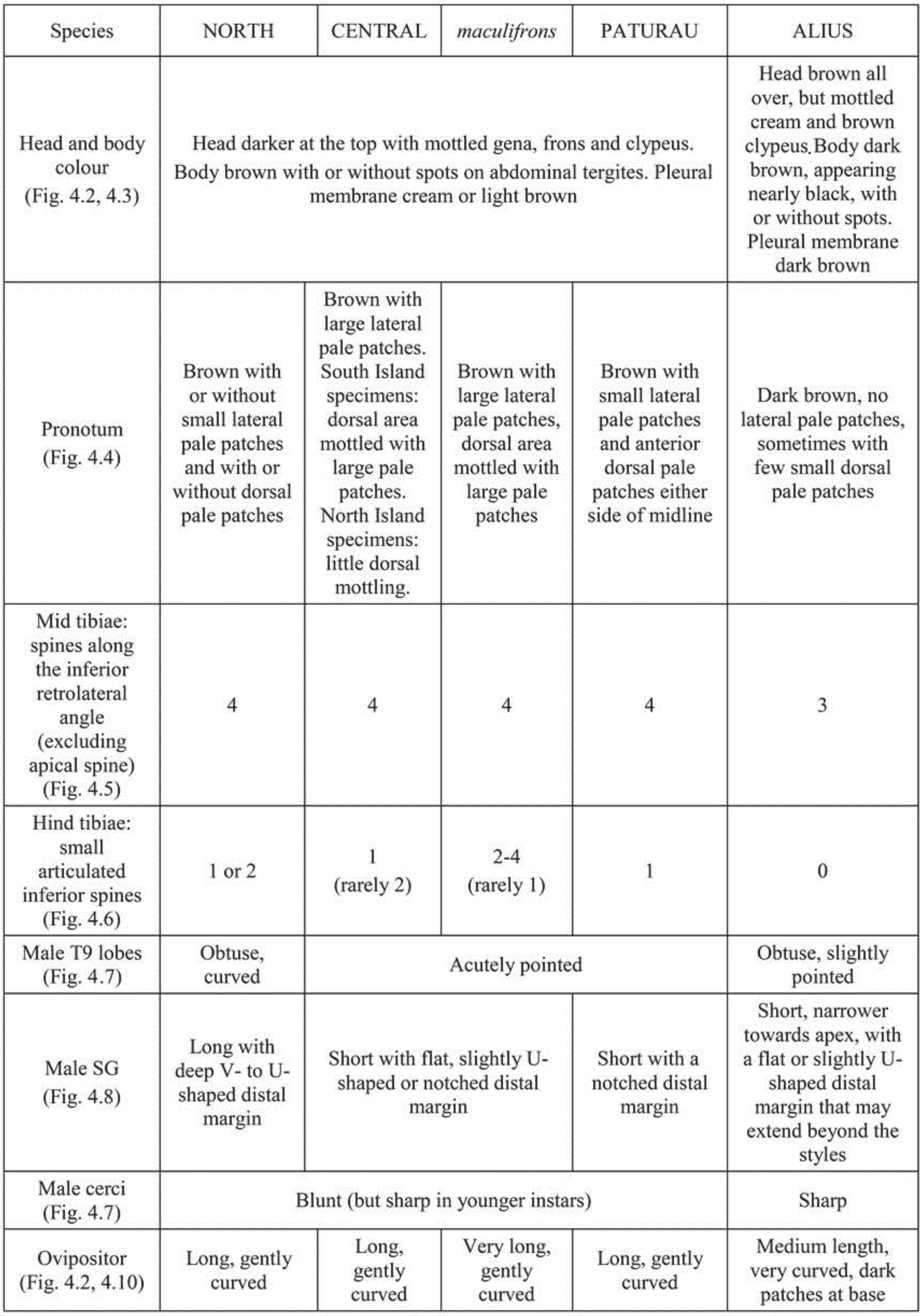
[4] Jewell (2007) observed that the fourth segment of the maxillary palps of *H. nitaweta* are pilose only on the distal 45%, but I have observed that MP4 of *H. nitaweta* are pilose along their entire length continuing on to MP3 (See chapter 7, Figure 7.5). Other than this discrepancy, all other features and the locality details match those of *H. nitaweta* as described by Jewell (2007), therefore the specimens that I have examined are unlikely to be of a different species.

be distinguished from the five species in this analysis by the absence of spots on its hind femora while *H*. “madisylvestris” has spots on its hind femora but is larger than all five species described here (Jewell 2007).

Table 4.2. Useful features for distinguishing between the five species included in this analysis.



Species	NORTH	CENTRAL	*maculifrons*	PATURAU	ALIUS
Head and body colour (Fig. 4.2, 4.3)	Head darker at the top with mottled gena, frons and clypeus. Body brown with or without spots on abdominal tergites. Pleural membrane cream or light brown				Head brown all over, but mottled cream and brown clypeus. Body dark brown, appearing nearly black, with or without spots. Pleural membrane dark brown
Pronotum (Fig. 4.4)	Brown with or without small lateral pale patches and with or without dorsal pale patches	Brown with large lateral pale patches. South Island specimens: dorsal area mottled with large pale patches. North Island specimens: little dorsal mottling.	Brown with large lateral pale patches, dorsal area mottled with large pale patches	Brown with small lateral pale patches and anterior dorsal pale patches either side of midline	Dark brown, no lateral pale patches, sometimes with few small dorsal pale patches
Mid tibiae: spines along the inferior retrolateral angle (excluding apical spine) (Fig. 4.5)	4	4	4	4	3
Hind tibiae: small articulated inferior spines (Fig. 4.6)	1 or 2	1 (rarely 2)	2-4 (rarely 1)	1	0
Male T9 lobes (Fig. 4.7)	Obtuse, curved	Acutely pointed			Obtuse, slightly pointed
Male SG (Fig. 4.8)	Long with deep V- to U-shaped distal margin	Short with flat, slightly U-shaped or notched distal margin		Short with a notched distal margin	Short, narrower towards apex, with a flat or slightly U-shaped distal margin that may extend beyond the styles
Male cerci (Fig. 4.7)	Blunt (but sharp in younger instars)				Sharp
Ovipositor (Fig. 4.2, 4.10)	Long, gently curved	Long, gently curved	Very long, gently curved	Long, gently curved	Medium length, very curved, dark patches at base

Appendix

Appendix 4.1. Specimens examined for morphological traits.

Code	Species	Location	Area Code	Sex
DOCORD048529B	ALIUS	Rotoroa	BR	F
DOCORD048677	ALIUS	Rotoroa	BR	F
DOCORD049133	ALIUS	Rotoroa	BR	F
DOCORD049316B	ALIUS	Rotoroa	BR	F
DOCORD049316C	ALIUS	Rotoroa	BR	F
DOCORD055878B	ALIUS	Huia Caves	NN	F
DOCORD055928	ALIUS	Mt Arthur	NN	F
DOCORD070144A	ALIUS	Lake Chalice	MB	F
DOCORD070144B	ALIUS	Lake Chalice	MB	F
GW10A	ALIUS	St Arnaud	BR	F
GW10BB	ALIUS	St Arnaud	BR	F
GW10BS	ALIUS	St Arnaud	BR	F
GW212	ALIUS	Mt Haidinger	NN	F
GW46	ALIUS	St Arnaud	BR	F
GW481	ALIUS	St Arnaud	BR	F
GW664	ALIUS	Moanui	BP	F
GW690	ALIUS	Flora Carpark	NN	F
GW692	ALIUS	Flora Carpark	NN	F
GW734	ALIUS	Waikaremoana	GB	F
GW76	ALIUS	Awakiri Valley	BR	F
GW834	ALIUS	Awatuna	WD	F
GW896C	ALIUS	Denniston	NN	F
GW899	ALIUS	Denniston	NN	F
GW918	ALIUS	Wairata	BP	F
GW94A	ALIUS	Waikaremoana	GB	F
DOCORD048036	ALIUS	Braeburn Track	BR	M
DOCORD048529A	ALIUS	Rotoroa	BR	M
DOCORD048529C	ALIUS	Rotoroa	BR	M
DOCORD055762	ALIUS	Bellbird Bush	HB	M
DOCORD056594	ALIUS	Cache's Bush	HB	M
DOCORD070144C	ALIUS	Lake Chalice	MB	M
DOCORD070144D	ALIUS	Lake Chalice	MB	M
DOCORD070144E	ALIUS	Lake Chalice	MB	M
GW33BB	ALIUS	Nelson Lakes	BR	M
GW33BS	ALIUS	Nelson Lakes	BR	M
GW691	ALIUS	Flora Carpark	NN	M
GW695	ALIUS	Flora Carpark	NN	M
GW796	ALIUS	Coromandel	CL	M
GW896A	ALIUS	Denniston	NN	M
GW896B	ALIUS	Denniston	NN	M
GW995	ALIUS	Paturau River	NN	M
GW100	CENTRAL	Rameka	NN	F

GW1013	CENTRAL	Lewis Pass	BR	F
GW1014	CENTRAL	Lewis Pass	BR	F
GW1015	CENTRAL	Lewis Pass	BR	F
GW1016	CENTRAL	Lewis Pass	BR	F
GW102	CENTRAL	Rameka	NN	F
GW105	CENTRAL	Rameka	NN	F
GW143	CENTRAL	Lewis Pass	BR	F
GW23	CENTRAL	Lewis Pass	BR	F
GW552	CENTRAL	Ruahine Ranges	RI	F
GW553	CENTRAL	Ruahine Ranges	RI	F
GW567	CENTRAL	Rameka	NN	F
GW568	CENTRAL	Rameka	NN	F
GW571	CENTRAL	Rameka	NN	F
GW574	CENTRAL	Rameka	NN	F
GW575	CENTRAL	Rameka	NN	F
GW577	CENTRAL	Rameka	NN	F
GW578	CENTRAL	Rameka	NN	F
GW661	CENTRAL	Ruahine Ranges	RI	F
GW761	CENTRAL	Kahuterawa Valley	WN	F
GW815	CENTRAL	Kahuterawa Valley	WN	F
GW817	CENTRAL	Kahuterawa Valley	WN	F
GW900	CENTRAL	Abel Tasman National Park	NN	F
GW916B	CENTRAL	Ruahine Ranges	RI	F
GW1010	CENTRAL	Lewis Pass	BR	M
GW1012	CENTRAL	Lewis Pass	BR	M
GW104	CENTRAL	Rameka	NN	M
GW565	CENTRAL	Rameka	NN	M
GW569	CENTRAL	Rameka	NN	M
GW570	CENTRAL	Rameka	NN	M
GW573	CENTRAL	Rameka	NN	M
GW662	CENTRAL	Ruahine Ranges	NN	M
GW759	CENTRAL	Kahuterawa Valley	WN	M
FD4	*maculifrons*	Sinbad Gully	FD	F
GW0912	*maculifrons*	Arthurs Pass	MC	F
GW1017	*maculifrons*	Lewis Pass	BR	F
GW1018	*maculifrons*	Lewis Pass	BR	F
GW1019	*maculifrons*	Lewis Pass	BR	F
GW1020	*maculifrons*	Lewis Pass	BR	F
GW1021	*maculifrons*	Lewis Pass	BR	F
GW1022	*maculifrons*	Lewis Pass	BR	F
GW1025	*maculifrons*	Lewis Pass	BR	F
GW1099	*maculifrons*	Southland	SL	F
GW119	*maculifrons*	Waipohatu Stream	SL	F
GW146	*maculifrons*	Otira	WD	F
GW147	*maculifrons*	Paparoa Ranges	BR	F
GW198	*maculifrons*	Takitimu Range	SL	F
GW201	*maculifrons*	Takitimu Range	SL	F

GW28	*maculifrons*	Te Anau	FD	F
GW32A	*maculifrons*	Tautuku, Catlins	SL	F
GW462A	*maculifrons*	Lewis Pass	BR	F
GW462B	*maculifrons*	Lewis Pass	BR	F
GW463A	*maculifrons*	Lewis Pass	BR	F
GW463B	*maculifrons*	Lewis Pass	BR	F
GW670	*maculifrons*	Mt Arthur	NN	F
GW758	*maculifrons*	Denniston	NN	F
GW788	*maculifrons*	Flora Saddle	NN	F
GW826	*maculifrons*	Awatuna	WD	F
GW827	*maculifrons*	Awatuna	WD	F
GW828	*maculifrons*	Awatuna	WD	F
GW829	*maculifrons*	Awatuna	WD	F
GW830	*maculifrons*	Awatuna	WD	F
GW832	*maculifrons*	Awatuna	WD	F
GW835	*maculifrons*	Awatuna	WD	F
GW836	*maculifrons*	Awatuna	WD	F
GW837	*maculifrons*	Awatuna	WD	F
GW861	*maculifrons*	Awatuna	WD	F
GW864	*maculifrons*	Denniston	NN	F
GW872	*maculifrons*	Rolleston Track	BR	F
GW873	*maculifrons*	Rolleston Track	BR	F
GW874	*maculifrons*	Rolleston Track	BR	F
GW875	*maculifrons*	Rolleston Track	BR	F
GW876	*maculifrons*	Rolleston Track	BR	F
GW877	*maculifrons*	Rolleston Track	BR	F
GW895	*maculifrons*	Denniston	NN	F
GW898	*maculifrons*	Arthurs Pass	MC	F
GW908	*maculifrons*	Arthurs Pass	MC	F
GW909B	*maculifrons*	Arthurs Pass	MC	F
GW910B	*maculifrons*	Arthurs Pass	MC	F
GW911	*maculifrons*	Arthurs Pass	MC	F
GW913F	*maculifrons*	Arthurs Pass	MC	F
GW914f	*maculifrons*	Arthurs Pass	MC	F
GW915f	*maculifrons*	Arthurs Pass	MC	F
GW922f	*maculifrons*	Kaiata	BR	F
GW923	*maculifrons*	Kaiata	BR	F
GW924f	*maculifrons*	Kaiata	BR	F
GW925f	*maculifrons*	Kaiata	BR	F
GW942	*maculifrons*	Lake Marion	NC	F
GW943	*maculifrons*	Lake Marion	NC	F
GW1011	*maculifrons*	Lewis Pass	BR	M
GW133	*maculifrons*	Craigieburn Forest Park	MC	M
GW141	*maculifrons*	Kepler Track	FD	M
GW142	*maculifrons*	Fiordland	FD	M
GW461	*maculifrons*	Lewis Pass	BR	M
GW824	*maculifrons*	Awatuna	WD	M

GW825	*maculifrons*	Awatuna	WD	M
GW831	*maculifrons*	Awatuna	WD	M
GW840	*maculifrons*	Awatuna	WD	M
GW909A	*maculifrons*	Arthurs Pass	MC	M
GW910M	*maculifrons*	Arthurs Pass	MC	M
GW915M	*maculifrons*	Arthurs Pass	MC	M
GW920	*maculifrons*	Kaiata	BR	M
GW921	*maculifrons*	Kaiata	BR	M
GW922M	*maculifrons*	Kaiata	BR	M
AMNZ5630F	NORTH	Northland	ND	F
AMNZ5630FB	NORTH	Northland	ND	F
DOCORD047457	NORTH	Moki	TK	F
DOCORD048177A	NORTH	Opouri	SD	F
GW126	NORTH	Pureora	TO	F
GW169	NORTH	Maungatautari	WO	F
GW172	NORTH	Maungatautari	WO	F
GW195F	NORTH	Opepe	TO	F
GW196	NORTH	Opepe	TO	F
GW21	NORTH	Wairata	BP	F
GW223	NORTH	Ohauora	TK	F
GW224	NORTH	Ohauora	TK	F
GW234	NORTH	Coromandel	CL	F
GW247A	NORTH	Coromandel	CL	F
GW4	NORTH	Manganuku Bridge	BP	F
GW44	NORTH	Opononi	ND	F
GW464	NORTH	Ngaiotonga Reserve	ND	F
GW49A	NORTH	Puketi	ND	F
GW49B1	NORTH	Puketi	ND	F
GW49B2	NORTH	Puketi	ND	F
GW549	NORTH	Pureora	TO	F
GW554	NORTH	Atene	WI	F
GW557	NORTH	Atene	WI	F
GW624	NORTH	Pureora	TO	F
GW627	NORTH	Pureora	TO	F
GW628	NORTH	Pureora	TO	F
GW630	NORTH	Pureora	TO	F
GW631	NORTH	Pureora	TO	F
GW633	NORTH	Pureora	TO	F
GW636	NORTH	Atene	WI	F
GW64	NORTH	Manginangina Reserve	ND	F
GW665	NORTH	Moanui	BP	F
GW700	NORTH	Wairata	BP	F
GW703	NORTH	Wairata	BP	F
GW726	NORTH	Herekino Forest	ND	F
GW740	NORTH	Waikaremoana	GB	F
GW741	NORTH	Waikaremoana	GB	F
GW742	NORTH	Waikaremoana	GB	F

GW744	NORTH	Waikaremoana	GB	F
GW745	NORTH	Waikaremoana	GB	F
GW746	NORTH	Waikaremoana	GB	F
GW751	NORTH	Waikaremoana	GB	F
GW760	NORTH	Kahuterawa Valley	WN	F
GW765	NORTH	Kahuterawa Valley	WN	F
GW766	NORTH	Kahuterawa Valley	WN	F
GW777	NORTH	Lucy's Gully	TK	F
GW778	NORTH	Lucy's Gully	TK	F
GW797	NORTH	Kahuterawa Valley	WN	F
GW798	NORTH	Kahuterawa Valley	WN	F
GW799	NORTH	Kahuterawa Valley	WN	F
GW800	NORTH	Kahuterawa Valley	WN	F
GW801	NORTH	Kahuterawa Valley	WN	F
GW802	NORTH	Kahuterawa Valley	WN	F
GW803	NORTH	Kahuterawa Valley	WN	F
GW804	NORTH	Kahuterawa Valley	WN	F
GW805	NORTH	Kahuterawa Valley	WN	F
GW806	NORTH	Kahuterawa Valley	WN	F
GW807	NORTH	Kahuterawa Valley	WN	F
GW808	NORTH	Kahuterawa Valley	WN	F
GW809	NORTH	Kahuterawa Valley	WN	F
GW810	NORTH	Kahuterawa Valley	WN	F
GW811	NORTH	Kahuterawa Valley	WN	F
GW819	NORTH	Kahuterawa Valley	WN	F
GW893F	NORTH	Dawson Falls, Mt Taranaki	TK	F
GW90	NORTH	Waikaremoana	GB	F
GW919	NORTH	Mangamahoe	TK	F
GW937	NORTH	Tauranga	BP	F
GW944A	NORTH	Opotiki	BP	F
GW944B	NORTH	Opotiki	BP	F
AMNZ5630M	NORTH	Northland	ND	M
GW218	NORTH	Raurimu	TO	M
GW219	NORTH	Raurimu	TO	M
GW220	NORTH	Raurimu	TO	M
GW222	NORTH	Ohauora	TK	M
GW225	NORTH	Ohauora	TK	M
GW229	NORTH	Lucy's Gully	TK	M
GW239	NORTH	Taranaki	TK	M
GW548	NORTH	Pureora	TO	M
GW550	NORTH	Pureora	TO	M
GW551	NORTH	Pureora	TO	M
GW558	NORTH	Atene	WI	M
GW559	NORTH	Atene	WI	M
GW625	NORTH	Pureora	TO	M
GW68	NORTH	Waikaremoana	GB	M
GW735	NORTH	Waikaremoana	GB	M

GW738	NORTH	Waikaremoana	GB	M
GW739	NORTH	Waikaremoana	GB	M
GW74	NORTH	Auckland	AK	M
GW753	NORTH	Waikaremoana	GB	M
GW767	NORTH	Kahuterawa Valley	WN	M
GW768	NORTH	Kahuterawa Valley	WN	M
GW769	NORTH	Kahuterawa Valley	WN	M
GW770	NORTH	Kahuterawa Valley	WN	M
GW771	NORTH	Kahuterawa Valley	WN	M
GW772	NORTH	Kahuterawa Valley	WN	M
GW773	NORTH	Kahuterawa Valley	WN	M
GW774	NORTH	Kahuterawa Valley	WN	M
GW775	NORTH	Kahuterawa Valley	WN	M
GW812	NORTH	Kahuterawa Valley	WN	M
GW813	NORTH	Kahuterawa Valley	WN	M
GW814	NORTH	Kahuterawa Valley	WN	M
GW944M	NORTH	Opotiki	BP	M
GW984	PATURAU	Arorere Shelter	NN	F
GW27	PATURAU	Paturau River	NN	M

Appendix 4.2. Additional specimens were examined from these locations in order to map species' distributions.

Species	Locations	Source
ALIUS	Pureora, TO; Harwoods Hole, NN; Canaan Road, NN; Cobb Valley, NN; Oparara Basin, NN; Lake Hanlon, NN; Lewis Pass, BR; Brooklyn Valley, NN; Porika Track, BR; Shanandoah Saddle, BR; Wairau Valley, MB; Maruia Saddle Road, BR; Rakaroa Scenic Reserve, GB; Whirinaki, TO; Whakapunake, GB.	NZAC, GW, NZAM, DOC
CENTRAL	Huiarau Range, BP; Pouakai Hut, Mt Taranaki, TK; Dawson Falls, Mt Taranaki, TK; Herepai Hut, Tararua Ranges, WN; Rimutaka Range, WN; Maungapohatu Road, GB; Makahu Saddle, HB; Canaan Road, NN; Cobb Lookout, NN.	NZAC, GW, DOC
maculifrons	Mt Sewell Track, BR; Te Kuha, NN; Braeburn Track, BR; Mahitahi River, WD; Piano Flat, CO; Hollyford Valley, OL; Wet Jacket Arm, FD; Bowen Falls, FD; Secretary Island, FD; Franz Josef, WD; Barrier Valley, WD; Homer Saddle, FD; Leith Saddle Track, DN; Haast, WD; Karangarua River, WD; Hunts Beach, WD; Methven, MC; Callery Gorge Track, WD; Mt Algidus, MC; Wanganui River, WD; Ross, WD; Lake Hauroko, FD; Dean Burn, FD; Borland Saddle, FD; West Arm,Manapouri, FD; Lake Paringa, WD; Waita River, WD; Okarito, WD; Mt Owen, NN; Tasman Wilderness Area, NN; Haldane Reserve, SL; Eglinton Valley, OL.	GW, DOC, NZAC, FD,
NORTH	Waiwera, AK; Te Puia Lodge, Kaweka Forest Park, HB; Pelorus, SD; Waimakau, AK; Matuku Reserve, AK; Kaipara, AK; Moehau Range, CL; Omahuta Kauri Reserve, ND; Red Hills Track, MB; Waipoua Forest, ND; Waitewhena Forest, TK; Mataitai Forest, AK; Jackson's Lookout, Mt Taranaki, TK; Whangamomona Saddle, TK; Owhango, TO; Tiekekaianga, RI; Paengaroa, RI; Orangihikoia,GB; Rahui Island, GB; Huiarau Summit, GB; Maraunui, GB; Tarewara, GB; Esson Valley, SD; Meeting of the Waters Scenic Reserve, TK; Makino Forest, TK.	GW, NZAC, NZAM, DOC

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Chapter 5. Assessment of the validity of *Hemiandrus* tag-names; data enrichment to serve Department of Conservation needs

Introduction

Species are separately evolving metapopulation lineages (de Queiroz 1998) and are the only natural groupings of taxa. Genera and other higher ranks should, where possible, reflect monophyly and evolutionarily distinctiveness, but they are generally accepted as arbitrary units (Vences *et al.* 2013). As a result, genera can vary in size from monotypy to consisting of thousands of species. Differences in genus size may be biologically real, reflecting factors such as differences in genus age (Raup *et al.* 1973), or variation in extinction rates and/or speciation rates. Differences might alternatively be artefacts of classification criteria in vogue during the time at which the genus was erected (Walters 1961), taxonomic error, or deliberate attempts to maximise taxonomic recognition of variation in order to conserve biodiversity.

Although New Zealand is a continental island, the composition of its biota is much more like that of an oceanic island in that it is characterised by stochasticity associated with the random arrival and extinction of lineages resulting in some depauperate lineages and some speciose lineages (Goldberg *et al.* 2008; Trewick *et al.* 2007). For example, in New Zealand there is an abundance of anostostomatid and raphidophorid orthopterans but relatively few Acrididae (grasshoppers) and Gryllidae (crickets) and no endemic Gryllacrididae (Trewick and Morgan-Richards 2014). Lineages within New Zealand Anostostomatidae also appear to express this pattern; five genera form at least three lineages: tree (*Hemideina*) and giant wētā (*Deinacrida)*, tusk wētā (*Motuweta*, *Anisoura*) and ground wētā (*Hemiandrus*) that combined, do not form a clade and thus appear to be a subset of possible diversity (Pratt *et al.* 2008).

Hemiandrus (Ander 1938) appears to be the most speciose of the New Zealand anostostomatid genera, with estimates suggesting it comprises 35 species (Gordon 2010). Other New Zealand anostostomatid genera comprise between one (*Anisoura*) and eleven (*Deinacrida)* species. This high diversity in *Hemiandrus* relative to other New Zealand anostostomatid species may be due to life-history properties of *Hemiandrus* that increase speciation rates or decrease extinction rates, an older age of the *Hemiandrus* lineage or inaccurate taxonomy. To date, only eleven *Hemiandrus* species have been formally described (Jewell 2007; Johns 1997; Johns 2001; Taylor Smith *et al.* 2013 (chapter 6)) and an additional four species were described in chapter 4 (Table 5.1), but approximately 31 putative species currently have tag-names (Department of Conservation 2010; Jewell 2007; Johns 2001). A tag-name is an informal name that indicates an entity that may be a separate species, monophyletic group or separate interbreeding population of uncertain taxonomic rank (Leschen *et al*. 2009). Some of these tag-names have been referred to in the peer-reviewed literature (e.g. Jewell (2007), Trewick *et al.* (2012) Taylor Smith *et al*. (2013)).Tag-names assigned to putative taxa are not subject to International Codes of Nomenclature (iczn.org) and therefore represent only observations of morphological variation. A problem with tag-names is that associated data is minimal so that it is unclear which biological entities they represent (Leschen *et al.* 2009). Furthermore, the use of tag-names can result in many different references to the same organism. For example *Hemiandrus* “horomaka” (Johns 2001) and *H*. “peninsularis” (Wahid 1978) likely refer to the same entity (Gwynne 2004). Therefore the apparently high species diversity in *Hemiandrus* might be an artefact of insufficient taxonomic analysis.

The taxonomic validity of proposed ground wētā species diversity has been tested in only one instance. Johns (2001) noted the presence of morphological variation within the widespread North Island ground wētā, *H. pallitarsis*. Chappell *et al.* (2012) showed that *H. pallitarsis* had high mtDNA diversity across its range but a lack of concordant variation in morphological, genetic and acoustic traits supported its treatment as a single species. No other ground wētā have been thoroughly tested using multiple lines of evidence in this way. Here I use mtDNA sequence data to supplement our taxonomic knowledge and explore the putative diversity within *Hemiandrus*: to what extent do tag-names reflect biological diversity within this genus? Mitochondrial DNA sequence data in conjunction with morphology is a powerful tool to test hypotheses of species, using the genotypic cluster definition (Mallet 1995). This predicts concordance of characters and is independent of the process of speciation. Where mtDNA sequence data supports distinct clades and genetic distinctiveness concordant with morphological traits distinguishing putative species then these species will be recognised here.

Table 5.1. *Hemiandrus* appears to be the most speciose of the New Zealand anostostomatid genera although only eleven *Hemiandrus* species have been formally described to date.

Species name	Authority	Previously known by	Species status supported?
maculifrons	Walker, 1869	*gracilis* Salmon, 1950	Yes (see chapter 3)
pallitarsis	Walker, 1869	*furcifer* Ander, 1938	Yes, see Chappell *et al.* (2012)
focalis	Hutton, 1897	*maculifrons* sensu Salmon, 1950	Not mutually exclusive with respect to *H. superba*
bilobatus	Ander, 1938	NA	Yes
fiordensis	Salmon, 1950	NA	Not tested because DNA could not be amplified
subantarcticus	Salmon, 1950	NA	Yes
lanceolatus	Walker, 1869	NA	*Nomen dubium*
superba	Jewell, 2007	NA	Possibly synonymous with *H. focalis*
nitaweta	Jewell, 2007	NA	Yes
maia	Taylor Smith *et al.*, 2013	"evansae"	Yes
electra	Taylor Smith *et al.*, 2013	"okiwi"	Likely comprises more than a single species
ALIUS[1]	Chapter 4	NA	Likely comprises more than a single species
NORTH[1]	Chapter 4	NA	Yes (see chapter 3)
PATURAU[1]	Chapter 4	NA	Yes
CENTRAL[1]	Chapter 4	NA	Yes (see chapter 3)

Methods

Collection and identification of specimens

Ground wētā specimens were obtained from Department of Conservation (DoC) pitfall traps (Sinclair and Stringer 2003), New Zealand Arthropod Collection (NZAC), Auckland Museum of New Zealand (NZAM), specimens collected by Eric Edwards (DoC), Tony Jewell and the Phoenix Lab insect collection at Massey University, Palmerston North. Additional specimens were collected from four sites in summer 2013-2014 specifically for this study and submitted to the Phoenix Lab collection: Dodson Valley, Nelson; Pureora Forest Park; Red Hills Ridge, Mt Richmond Forest Park; Mt Tapuae-o-Uenuku, Inland Kaikoura Range. These four sites were targeted because tag-named *Hemiandrus* with location names are currently considered to be restricted to these areas, and appear in DoC threat lists (Department of Conservation 2010; Johns 2001; Trewick *et al.* 2012).

Phoenix Lab ground wētā (codes with GW prefix) were collected between 1990 and 2014 and preserved in 95% ethanol (Moreau *et al.* 2013). Other specimens were either dry or preserved in 70-95% ethanol.

Specimens representing tag-named taxa were identified using the following traits: maxillary palp pilosity; the number and positioning of superior spines on the fore and hind tibiae; the shape of male and female terminalia; tarsus pilosity and colour; adult body size; texture of the frons (see Johns 2001; Jewell 2007; pers obs). Described species were identified based on formal species descriptions. The number of stridulatory pegs and antennomeres (Johns 2001) were not used for identification as these traits were found to be more variable than specified and often overlapping between species. Geography was not used to identify species as ongoing study has revealed many taxa have wider ranges than initially proposed.

DNA extraction, amplification & sequencing

Muscle tissue from a hind femur of each specimen was dissected out and subject to total genomic DNA extraction using a salting-out method (Sunnucks and Hales 1996). A fragment of the mitochondrial cytochrome oxidase I gene (COI) was amplified (primers details see chapter 3). Polymerase chain reaction (PCR) used the following conditions: 94°C for 2 min then 35 cycles of 94°C for 30s, annealing at 55°C for 30s, and extension at 72°C for 1m, followed by 72°C for 8 min. Amplified DNA products were treated to Shrimp Alkaline Phosphotase⁄Exonuclease digestion (USB Corp.) to remove unincorporated primers prior to sequencing. Cycle sequencing with the PCR primers used Bigdye v3.1 chemistry (PE) following the manufacturer’s protocols, with automated reading on an ABI3730. Sequences were edited and aligned using Geneious v6.1.6 (Kearse *et al.* 2012).

Phylogenetic analysis

Phylogenetic analysis of the COI alignment (*n* = 94 *Hemiandrus* individuals, Appendix 5.1) used a homologous sequence from the tree wētā *Hemideina crassidens* as the outgroup. To determine the most appropriate model of DNA evolution, log-likelihood scores were generated and compared using the Akaike information criterion (AIC) test in jModelTest v2.1.3 (Darriba *et al.* 2012). Bayesian phylogenetic analysis was performed using a general time reversible model of molecular evolution incorporating a proportion of invariant sites and a gamma distribution (GTR+I+G model) in MR BAYES v3.1.2 (Huelsenbeck and Ronquist 2005) as implemented in Geneious v6.1.6. The analysis was performed with two independent simultaneous runs for six million generations with three heated chains that were sampled every 6000 generations. Consensus tree and credibility values for each node were obtained after removal of burnin trees (10% of trees) after examination of output statistics using TRACER (Rambaut and Drummond 2009). Phylogenetic analyses were also performed using the maximum-likelihood (ML) method (Felsenstein 1981) in RAxML (Stamatakis 2006). Additionally, statistical parsimony analyses (TCS) (Templeton *et al.* 1992) were conducted with the COI sequences of lineages with ambiguous relationships to create haplotype networks using Population Analysis with Reticulate Trees (PopART: popart.otago.ac.nz).

Morphospecies were tested for phylogenetic parameters associated with species boundaries including monophyly and genetic distinctiveness. Phylogenetic relationships were considered strongly supported in Bayesian analysis when posterior probabilities were ≥0.95 and in the ML analysis when bootstrap proportions were ≥70% (Alfaro *et al.* 2003; Hillis and Bull 1993). In chapter 3 I observed that "species delimitation" statistics generated in Geneious using the species delimitation plugin (Masters *et al.* 2011) were not effective at delimitating putative species within *Hemiandrus.* I also found that genetic distances calculated based on the phylogeny varied depending on the specimens included, the model used and the method of tree construction. Therefore only mean within and between clade genetic K2P-corrected genetic distances were calculated using MEGA v6.0 (Kimura 1980; Tamura *et al.* 2013). From these distances I calculated the intra/inter ratio. Some lineages with high intraspecific genetic distances or comprising multiple well-supported clades were split into subclades and genetic distances were calculated.

Results

Concordance supports named and tag-named lineages

Twenty-four putative taxa were included in the phylogenetic analysis (ten with tag-names, nine of eleven described species, four species described in chapter 3 and one species whose morphology did not match any names (Table 5.1, 5.2). Absent from the analysis, because mtDNA could not be amplified or specimens were not available, were *H. fiordensis* and *H. lanceolatus*.

I tested morphospecies for phylogenetic parameters associated with species boundaries. The dataset comprised cytochrome oxidase 1 sequences from 94 *Hemiandrus* individuals and a single *Hemideina crassidens* sequence as the root species. The alignment of 797 bp, consisted of 162 (20%) variable, and 155 (19%) parsimony informative positions in the ingroup. The Bayesian and ML mtDNA phylogenies of 94 *Hemiandrus* specimens (Fig. 5.1) were largely concordant and supported many described and tag-named taxa as genotypic clusters and separately evolving lineages (Table 5.1, 5.2). The following 15 morphospecies formed clades in both the Bayesian and ML trees and were well-supported (Table 5.3): *H.* ALIUS, *H.* NORTH, *H.* CENTRAL, *H. maculifrons*, *H. nitaweta*, *H. subantarcticus*, *H.* “disparalis”, *H.* “elegans”, *H. maia*, *H.* “hapuku”, *H.* “furoviarius”, *H.* “timaru”, *H.* “vicinus”, *H.* “onokis” and *H.* “horomaka”. For these 15 putative species I found concordance of the diagnostic morphological traits as presented with their tag-names (Johns 2001) and their mtDNA data. In many instances the details by provided by Johns (2001) were sufficient to differentiate a tag-named entity from all others. For example the combination of paired pits on the female sternite, a long ovipositor, bare third maxillary palp segments (MP3) and fore-/midtibial spine numbers set *H.* “hapuku” apart from all other tag-named species. In other instances morphological data were insufficient. For example *H.* “disparalis” and *H.* “elegans” both have long ovipositors, bare MP3, and the same number of fore-/midtibial spines (Johns 2001). Other details that were said to differentiate the two (antennomere number) overlapped too much to be useful. However, I did find that differences in male terminalia existed among individuals with traits of *H.* “disparalis” and *H.* “elegans” and so used these to assist in species discrimination.

Table 5.2. Tag-named *Hemiandrus* taxa. All have some locality data provided. Names have been proposed by various authors and have been coded to indicate this: A=Johns (2001); B= Johns (date unknown); C=Wahid (1978); D=Jewell (2007). * Some tag-names not on the list because they are not of conservation concern, while others are yet to be included. **Tag-names with no associated date were included as they are present on the DoC threat list. †"timaru" and "porters" are not distinguishable based on the morphological details given so are treated here as a single tag-name: "timaru".

Tag-name	Author	Some morphological details given?	On DoC Orthoptera threat list?*	DNA tested here	Species status supported?
"disparalis"	A	Y	Y	Y	Yes
"dodsons"	A	N	Y	N	Locality associated with tag-name searched but no new taxa found
"elegans"="moehau"	A/B **	Y	Y	Y	Yes
"furoviarius"	A	Y	Y	Y	Yes
"hapuku"	A	Y	Y	Y	Yes
"peninsularis" ="horomaka"	C/A	Y	Y	Y	Yes but may comprise more than a single species
"kapiti"	A	Y	Y	Y	Yes. Specimens are found on Kapiti Island and match no other tag-name, however they do not match all morphological details provided in Johns (2001).
"madisylvestris"	A	Y	N	Y	Yes
"mtgeorge"	A	N	Y	N	Specimens not available
"nokomai"	A	Y	Y	N	Specimens not available
"onokis"	A	Y	Y	Y	Yes
"otautau"	A	N	N	N	Specimens not available
"otekauri"	A	Y	Y	N	Specimens not available
"promonotorius" ="cape campbell"	A/B	Y	Y	Y	No. Synonymous with *H. bilobatus*
"pureora 1"	A	N	Y	N	Locality associated with tag-name searched but no new taxa found
"pureora 2"	A	N	Y	N	Locality associated with tag-name searched but no new taxa found
"redhills"	A	N	Y	N	Locality associated with tag-name searched but no new taxa found
"richmond"	A	N	Y	N	Specimens not available
"saxatilis"	A	Y	Y	N	Not tested because DNA could not be amplified, but is morphologically distinct
"staveley"	A	N	Y	N	Specimens not available
"timaru"	A	Y	Y	Y †	Yes
"porters"	A	Y	Y		
"turgidulus"	A	Y	N	N	Specimens not available
"waimakariri"	A	N	Y	N	Specimens not available
"vicinus"	A	Y	Y	Y	Yes
"cromwell"	B	N	Y	N	Specimens not available
"longwood range"	B	N	Y	N	Specimens not available
"rocklands"	B	N	Y	N	Specimens not available
"tapuaenuku"	B	N	Y	N	Locality associated with tag-name searched but no new taxa found
"esperance valley"	D	Y	N	N	Specimens not available
"hunter mountains"	D	Y	N	N	Specimens not available

Hemiandrus PATURAU was represented by a single specimen so support for monophyly and concordance with morphology could not be assessed, but the mtDNA was well-differentiated (0.17) from its closest neighbour, *H. maculifrons*. *Hemiandrus* "madisylvestris" was represented by a single haplotype so its monophyly and support could not be assessed, but it was well-diverged (0.10) from its sister species, *H. nitaweta*, with which it formed a well-supported clade

mtDNA differentiation within and among putative taxa

Detailed analysis of *H. Pallitarsis* (Chappell *et al.* 2012), *H. maculifrons*, *H.* NORTH and *H.* CENTRAL (chapter 3), indicated that each is a distinct cohesive species despite genetic and morphological variation. These were therefore used to guide interpretation of genetic differentiation within and between other *Hemiandrus* lineages.

Many candidate taxa had intraspecific distances, interspecific distances and intra- to –interspecific ratios within the ranges of the four reference species; *H.* subantarcticus, *H.* "disparalis", *H.* "elegans", *H*. "madisylvestris", *H. nitaweta*, *H*. "onokis", *H*. "vicinus", *H. maia*, *H.* "hapuku", *H.* PATURAU. *Hemiandrus* "timaru" and *H.* "furoviarius were closely related (0.08) compared to reference statistics (0.09-0.17) (Fig. 5.2), but their intra/inter ratio were within the reference range. Furthermore, the two putative species have obvious morphological differences; *H*. "timaru" can be distinguished by much longer ovipositors than *H.* "furoviarius". *Hemiandrus* "porters" could not be distinguished from *H.* "timaru" using the morphological details given in Johns (2001) (Table 5.4), so here the tag-name *H.* "timaru" may represent either or both species. However, the two specimens used were from areas consistent with existing estimates of the distribution of *H*. "timaru" and not *H*. "porters".

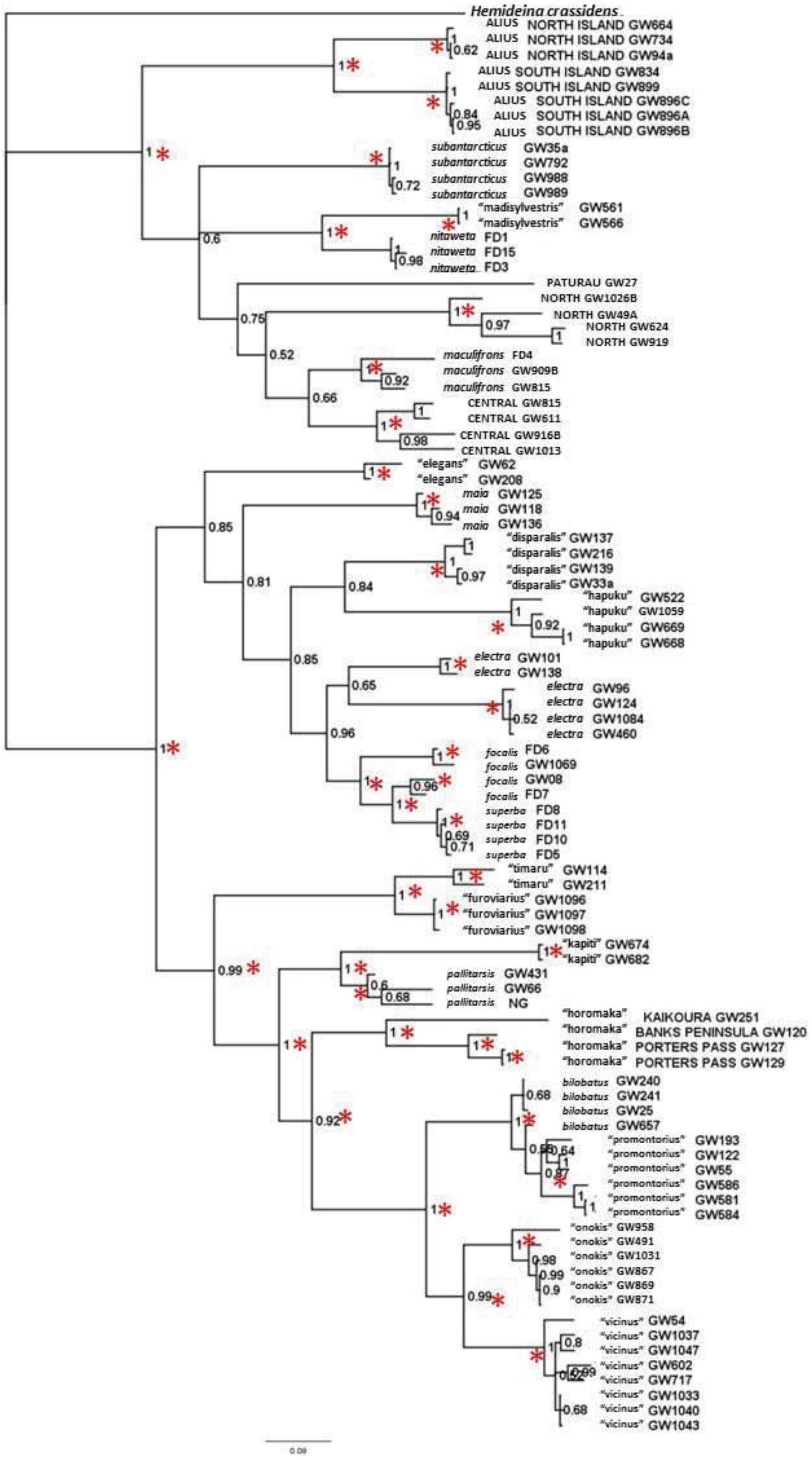

Figure 5.1. Bayesian COI mtDNA phylogeny of formally described and tag-named *Hemiandrus* taxa. Numbers at nodes represent Bayesian posterior probabilities, * indicates maximum likelihood bootstrap values >70%. Tag-names are given in inverted commas. New species proposed in this thesis are given in capitals. GW codes indicate accession in the Phoenix collection. Site localities are given for *H.* ALIUS and *H.* "horomaka".

Table 5.3. Clade support values, monophyly and parameters associated with species boundaries for all species included in the analysis. Grey rows indicate reference species; species supported by multiple lines of evidence as single species. * identical sequences

Species	n	Monophyly (ML)	Monophyly (Bayesian)	Clade Support (ML)	Clade support (Bayesian)	Closest Species	Mean Intra Distance (K2P)	Inter Dist (K2P)- Closest species	Intra/Inter ratio
pallitarsis	3	yes	yes	81	0.6	"kapiti"	0.05	0.12	0.44
maculifrons	3	yes	yes	96	1	CENTRAL	0.04	0.09	0.46
CENTRAL	4	yes	yes	96	1	*maculifrons*	0.04	0.09	0.45
NORTH	4	yes	yes	100	1	*maculifrons*	0.06	0.17	0.37
ALIUS	8	yes	yes	100	1	*nitaweta*	0.05	0.15	0.36
ALIUS (North Island)	3	yes	yes	100	1	ALIUS (South Island)	0.01	0.10	0.06
ALIUS (South Island)	5	yes	yes	100	1	ALIUS (North Island)	<0.01	0.10	0.03
nitaweta	3	yes	yes	100	1	"madisylvestris"	<0.01	0.10	<0.01
"madisylvestris"	2*	NA	NA	NA	NA	*nitaweta*	NA	0.10	NA
PATURAU	1	NA	NA	NA	NA	*maculifrons*	NA	0.17	NA
subantarcticus	4	yes	yes	100	1	*maculifrons*	<0.01	0.15	<0.01
"disparalis"	4	yes	yes	100	1	*focalis*	0.02	0.09	0.23
electra	6	yes	yes	45	0.65	*focalis*	0.07	0.10	0.69
electra (clade 1)	2	yes	yes	100	1	*focalis*	0.02	0.11	0.19
electra (clade 2)	4	yes	yes	100	1	*electra* (clade 1)	0.01	0.11	0.08
"elegans"	2	yes	yes	100	1	*maia*	0.02	0.16	0.12
focalis	4	no	no	NA	NA	*superba*	0.06	0.05	1.15
focalis (clade 1)	2	yes	yes	100	1	*focalis* (clade 2)	0.03	0.06	0.51
focalis (clade 2)	2	yes	yes	72	0.96	*superba*	0.07	0.04	1.82
focalis/superba	8	yes	yes	79	1	*electra*	0.05	0.10	0.51
superba	4	yes	yes	99	1	*focalis*	0.04	0.05	0.73
"hapuku"	4	yes	yes	100	1	"disparalis"	0.02	0.12	0.19
maia	3	yes	yes	100	1	*focalis*	0.02	0.13	0.16
bilobatus	4	yes	no	91	NA	"promontorius"	0.01	0.03	0.24
bilobatus/ "promontorius"	10	yes	yes	99	1	"onokis"	0.03	0.09	0.30
"promontorius"	6	yes	yes	41	0.87	*bilobatus*	0.03	0.03	0.80
"furoviarius"	3	yes	yes	100	1	"timaru"	<0.01	0.08	0.02
"horomaka" (including GW251-Kaikoura)	4	yes	yes	100	1	*pallitarsis*	0.07	0.15	0.47
"horomaka" (excluding GW251)	3	yes	yes	94	1	GW251-Kaikoura	0.02	0.12	0.19
GW251	1	yes	NA	NA	NA	"horomaka"	NA	0.12	NA
"kapiti"	2	yes	yes	100	1	*pallitarsis*	0.01	0.12	0.07
"onokis"	6	yes	yes	99	1	"vicinus"	0.02	0.10	0.23
"timaru"	2	yes	yes	95	1	"furoviarius"	0.03	0.08	0.33
"vicinus"	8	yes	yes	100	1	"onokis"	0.03	0.10	0.27

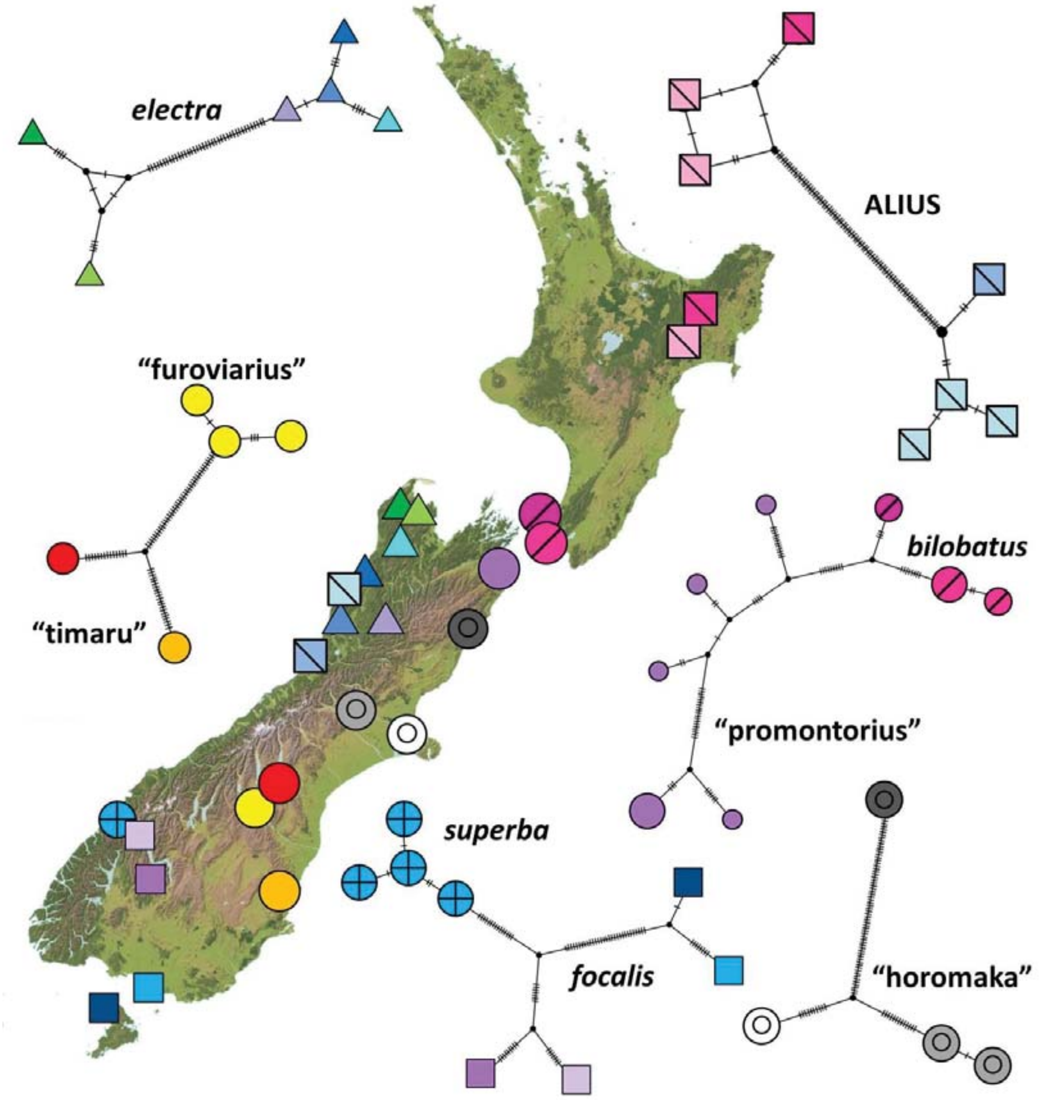

Figure 5.2. Haplotype networks of mtDNA (COI). Only species with ambiguous relationships, high genetic distances and high geographic structure are shown. *Hemiandrus* ALIUS, *H.* "electra" and *H.* "horomaka" had high genetic distances; *H. superba*/*H. focalis* and *H. bilobatus*/*H.* "promontorius" were not reciprocally monophyletic; *H.* "timaru" and *H.* "furoviarius" had relatively low interspecific genetic distances (0.08).

Table 5.4. Morphological overlap of *Hemiandrus* tag-named taxon pairs shows synonymy or trait ambiguity. Traits from Johns (2001).

Name	"porters"	"timaru"	*bilobatus*	"promontorius"
Pilosity of the third maxillary palp segment	Bare		Bare	
Fourth maxillary palp segment pilosity (%)	55%		55%	66%
Number of antennomeres	12-15	12-14	12	10
Superior fore tibial spines	1		2 (sometimes 1, pers obs)	2
Superior prolateral spines of mid tibiae	3		3	2 (sometimes 3, pers obs)
Superior retrolateral spines of mid tibiae	3		4	
Mid tibial symmetry	Asymmetrical, subapical missing		Symmetrical	
Tarsi	No details		Tarsi, few bare erect setae	
Stridulatory pegs	No details		1st-3rd tergites: 100-150 4th-6th: fewer	1st-4th tergites: 80-100
Male: margin of 8th tergite	Simple	No details	Large, bilobed	Bilobed, subtriangular
Male : edge of 9th tergite	Weakly produced, laterally thickened	No details	No details	Weakly produced, laterally thickened
Male: falci of 10th tergite	Wide	No details	Close	
Male: cerci	Blunt, setose	No details	Blunt, setose	
Female: 9th tergite	Simple		Simple	
Female: 6-7th sternite	Paired	Paired pits	Paired	Massive, bilobed
Female: cerci	Long, pointed, bare tip		Short, pointed, bare tip	Very short, bare, sharp
Female: ovipositor	Long		Short	

Three morphotaxa comprised highly divergent lineages suggesting that each might comprise more than a single species. *Hemiandrus* "horomaka" had a high mean intraspecific genetic distance when mtDNA sequences were compared (0.07). Instead of being endemic to Banks Peninsula and dry soils in the immediate vicinity (Johns 2001), specimens matching the morphology of *H.* "horomaka" were found in Christchurch, Banks Peninsula, Porters Pass and Kaikoura (Fig. 5.2). MtDNA from these specimens formed a well-supported clade with the Kaikoura haplotypes well-diverged and sister to other *H.* "horomaka" individuals; the mean interspecific distance among the two lineages (0.12) is consistent with that seen among species. None of the morphological features examined revealed differences concordant with this split. Likewise, *H. electra* had high distances (0.07) in the sample and comprised two well-supported clades; both in northwest Nelson (Fig. 5.2). The mean interspecific distance among the two clades (0.11) is consistent with that seen among species. The two clades may not be sister lineages as combined they had low clade support. Again, no morphological features examined revealed concordant variation. The *H.* ALIUS sample comprised two well-supported clades with disjunct distributions in North Island and South Island respectively (Fig. 5.2). The mean interspecific distance among the two clades (0.10) is consistent with that seen among species. No morphological differences were apparent between members of the two *H.* ALIUS clades.

Poorly resolved morphological differences & lack of mtDNA monophyly

Two pairs of morphospecies were not reciprocally monophyletic within the Bayesian mtDNA phylogeny (Fig. 5.1). *Hemiandrus bilobatus* was paraphyletic with respect to *H*. "promontorius" in the ML analysis. Although *H. bilobatus* is described from north of Cook Strait, it has been recorded on some of the Islands in the Marlborough Sounds while *H.* "promontorius" is found in Marlborough, South Island (Johns 2001) (Fig. 5.2). Morphological traits did not distinguish *H. bilobatus* and *H.* "promontorius" due to overlap or ambiguity (Fig. 5.3, Table 5.4) (Johns 2001). Together they formed a well-supported clade, but *H*. "promontorius" alone had poor node support (Bayesian 0.87, ML 41). The mean genetic distance within *bilobatus*/"promontorius" combined was 0.03, which is low compared to the reference species.

Hemiandrus focalis and *H. superba* were not reciprocally monophyletic. Rather, *H. focalis*, which is found throughout southern South Island, including Fiordland, and also on some offshore islands, was paraphyletic with respect to *H. superba*, which is restricted to Fiordland (Fig. 5.2). Together they formed a well-supported clade but also each of the two *H. focalis* clades and the *H. superba* clade were well-supported. The two putative species were not well-differentiated; mean genetic distance within *H. focalis* (0.06) was greater than the mean distance between *H. focalis* and *H. superba* (0.05). *Hemiandrus superba* also had a high intra- to interspecific ratio (0.73). The two species have been reported to be morphologically distinct in body size, colouration and rugosity (Jewell 2007), although *H. focalis* is variable in body size (Tony Jewell, pers comm; pers obs).

A single morphospecies collected from Kapiti Island matched no tag-name, but it formed a well-supported clade and was well-differentiated from its sister taxon, *H. pallitarsis*.

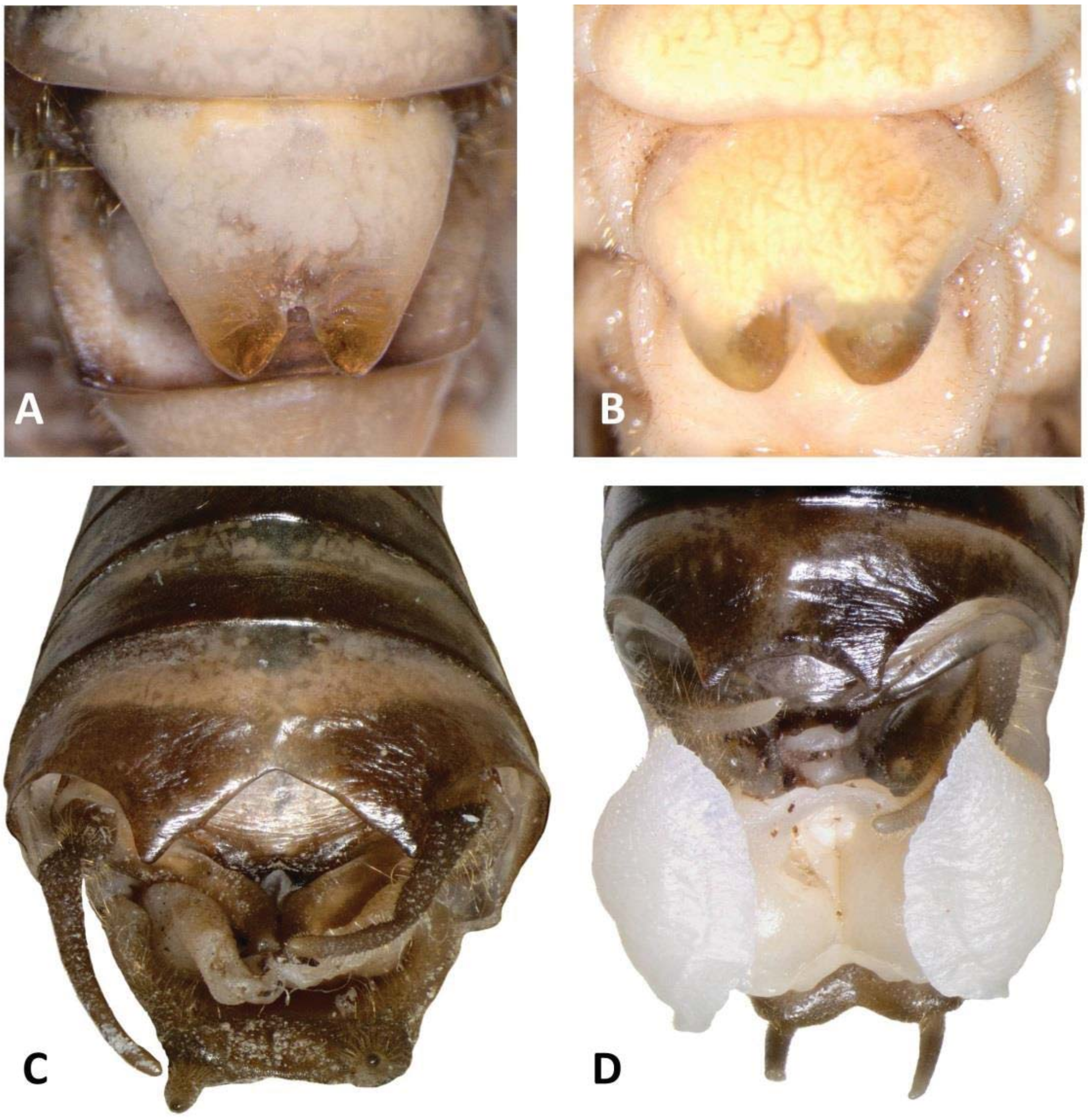

Figure 5.3. Similarity of two *Hemiandrus* morphospecies from either side of Cook Strait that are not reciprocally monophyletic. These taxa share the same arrangement of tibial spines and are similar in female 6th sternite shape (A. *Hemiandrus* "promontorius", B. *Hemiandrus bilobatus*) and male terminalia (C. *Hemiandrus* "promontorius", D. *Hemiandrus bilobatus*).

Locality-only tag-names

Four localities were searched for *Hemiandrus* because tag-named *Hemiandrus* with no morphological details were associated with them. At each site, representatives of at least one *Hemiandrus* species were found, although no specimens collected from these sites were morphologically or genetically distinct from ground wētā collected at one or more other sites and attributable to other names (Table 5.5).

Table 5.5. Localities of tag-named *Hemiandrus* searched during this study. * Specimens for which COI data are available.

Location	Expected tag-named species	Taxa present
Dodson Valley, Nelson	"dodsons"	"vicinus"*
Pureora Forest Park	"pureora 1"	NORTH*, ALIUS, *pallitarsis*
	"pureora 2"	
Red Hills Ridge, Mt Richmond Forest Park	"redhills"	"disparalis", *electra*, NORTH*, "onokis"*
Mt Tapuae-o-Uenuku, Inland Kaikoura Range	"tapuaenuku"	"hapuku"*

Distributions

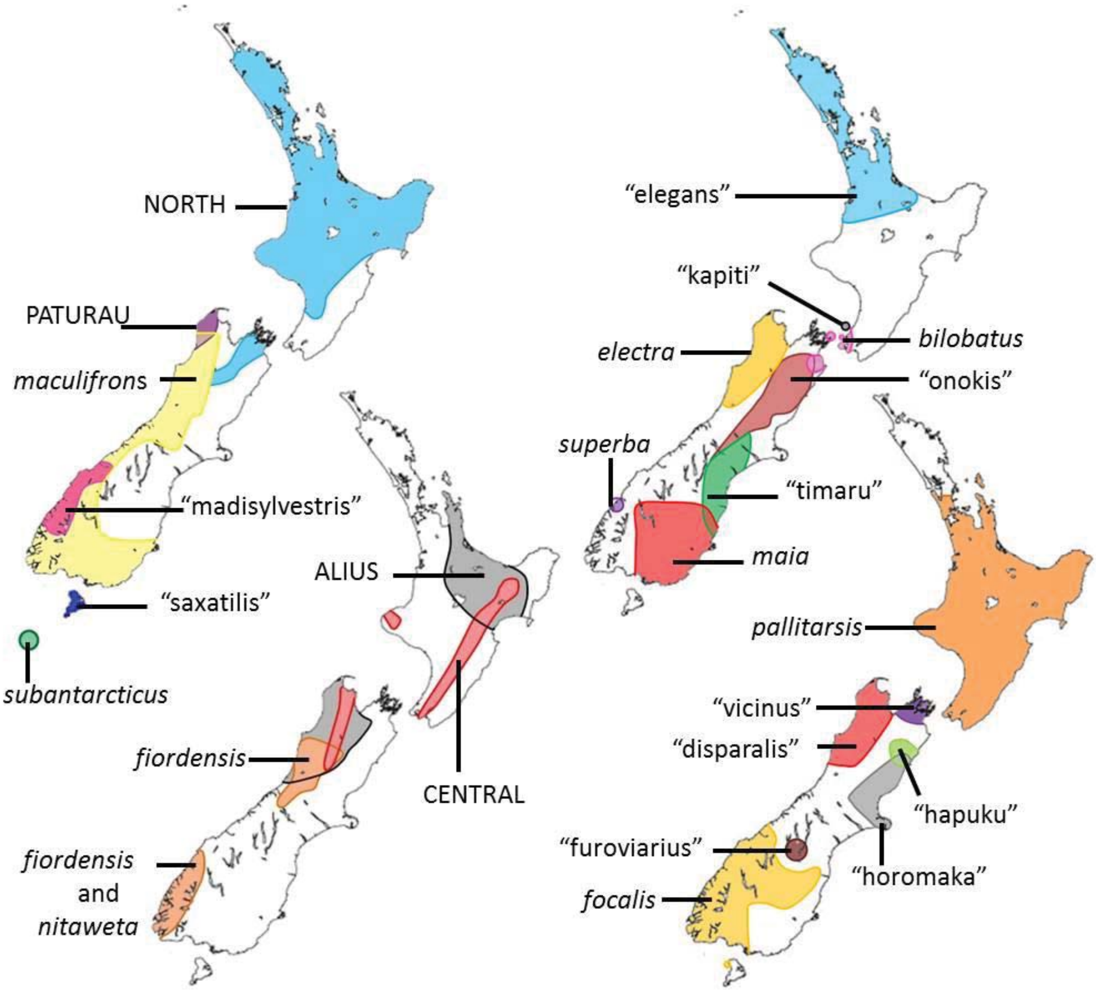

Figure 5.4. Updated distributions of *Hemiandrus*. Most morphospecies had wider ranges than previously documented (Jewell 2007; Johns 2001; Pratt *et al.* 2008). Note although *H. fiordensis* and *H.* "saxatilis" were not included in the phylogenetic analyses, their distributions have been included here as they are morphologically distinctive.

Most putative-species were shown here to have wider ranges than previously documented (Fig. 5.4, Appendix 5.2). For example *H.* "horomaka" was found in Christchurch, Banks Peninsula, Porters Pass and Kaikoura. The tag-name *H.* "elegans" was established based on specimens from the Moehau Range in the northern tip of Coromandel Peninsula and has since been recorded from a site near Rotorua (Department of Conservation 2010), but my observations indicate that this species is also found in Whareorino Forest (Waikato) and Puketi Forest (Northland). *Hemiandrus* "elegans" probably occupies suitable habitat throughout this region, but the fact that it is seldom found despite repeated searches suggests it occurs at low densities or is phenologically limited. All *H.* "onokis" included in this analysis have a more northerly distribution than previously illustrated (Johns 2001, Fig. 6) although specimens with the morphology of *H.* "onokis" from north of Kaikoura were reported (Johns 2001). In the present study *H.* "onokis" was found at Cape Campbell, Mt Richmond Forest Park, Renwick and upper and middle areas of the Clarence Valley. The maximum ranges of many taxa have not yet been discovered because some regions have not been thoroughly searched. For example *H.* NORTH and *H.* CENTRAL are likely to be found in suitable habitat in south east North Island.

Discussion

Hemiandrus has high species diversity

Hemiandrus species have received little attention in the scientific literature so basic details about their biology is lacking. Species distributions of described and putative species are poorly known with the possibility that some taxa might be in danger of extinction (Department of Conservation 2010). Until the taxonomy of the group is better understood the potential loss of species cannot be quantified.

I applied genetic analyses to *Hemiandrus* morphospecies to see whether the suggested high species diversity is real or simply an artifact of inaccurate taxonomy. Evidence presented here from mtDNA sequence data supports the inference that *Hemiandrus* does have high species diversity with a total of nine tag-names, eight described species and four species described in this thesis (chapter 3) being supported (Table 5.1, table 5.2). Three of these likely comprise more than a single species. *Hemiandrus* ALIUS and *H.* “horomaka” comprised multiple lineages separated by high genetic distances and might represent two species each (Fig. 5.1, Table 5.3). The two *H.* ALIUS clades appear to be restricted to North and South Islands respectively (Fig. 5.2) but no morphological differences were apparent. Likewise, *H. electra* might comprise two species, but the two lineages were not well-supported as sister species (Fig. 5.1). No morphological variation was observed between the two clades, both of which are found in northern South Island (Fig. 5.2).

Two names appear to represent geographic variation as two pairs of putative species were not reciprocally monophyletic (*H. superba/H. focalis*, *H.* “promontorius”/*H. bilobatus*). Under the phylogenetic species concept (Avise and Ball 1990; Baum and Shaw 1995; Donoghue 1985; Mishler 1985; Rosen 1979) lack of reciprocal monophyly would be evidence that they are not separately evolving lineages and therefore not distinct species. However, phylogenetic inference can be misled by mtDNA introgression and incomplete lineage sorting resulting in discordance between gene trees and species trees. As a result, species may not be reciprocally monophyletic on a tree inferred from mtDNA sequences. Instead of using monophyly as a defining feature, monophyly should be used as evidence of boundary support rather than a definition of species (Masters *et al.* 2011).

The low mean genetic distance found between *H. bilobatus* and *H.* “promontorius” (0.03) compared to distances found within other *Hemiandrus* species (e.g. 0.09-0.17 in the reference species) suggests that they might represent a single species. This inference is supported by the lack of reciprocal monophyly and the morphological similarity between the two. These two putative species are each range restricted and found either side of Cook Strait. Although some terrestrial taxa have ranges limits associated with the Cook Strait, many taxa have ranges that extend across Cook Strait to the southern tip of North Island (Wellington), likely due to the continuity of land between Marlborough and Wellington until recently (Trewick and Bland 2011). Therefore, along with the morphological similarities and absence of monophyly between the two, the treatment of *H. bilobatus* and *H.* "promontorius" as a single, recently disjunct species also makes more sense in light of New Zealand biogeography. Likewise, it appears that the lack of reciprocal monophyly between *H. focalis* and *H. superba* might also be explained by geographic variation within a single species. However, high morphological variation has been observed within *H. focalis* (Tony Jewell, pers comm & pers obs) which might suggest that the paraphyly of *H. focalis* with respect to *H. superba* could be the result of *H. focalis* comprising multiple species. Additional work is required.

One genetically distinct morphospecies did not match any of the existing tag-names, but Johns (2001) did tag-name a ground wētā from Kapiti Island, recording that females had long ovipositors. Intensive searching on several separate occasions for the present study failed to reveal any long-ovipositor females; all had very short ovipositors and matched *H.* "kapiti" in fore and mid tibial spine number.

Tag-names that were not investigated

Fifteen tag-names were not investigated due to a lack of material and/or a lack of detail about collection locality. Due to the inability to amplify DNA of *H. fiordensis* and the lack of *H. lanceolatus* specimens, these two described species were not included. However, *H. lanceolatus* is a nomen dubium as the holotype is a juvenile in poor condition (Johns 2001) and this species was reportedly found "in caves, half a mile within" (Walker 1869), habitat that is inconsistent with other ground wētā species. Intense searches were undertaken at the named locations of five other putative taxa. Although specimens of *Hemiandrus* were found at these places, none were morphologically or genetically distinct from other taxa. All of these individuals collected fitted the morphological details of other morphospecies (Table 5.5). This suggests that some tag-names may be the result of the over splitting of widespread species and these names probably mirror morphological variation within other tag-named species rather than new taxa.

Conclusion

Although the use of tag-names can be problematic, for some groups of organisms they represent the only taxonomic hypothesis on which one can attempt to conserve biodiversity or study natural history. In this analysis, I found most *Hemiandrus* tag-names and described species tested here appear to be separately evolving lineages, and can be defined as separate species based on Mallet's definition (1995). *Hemiandrus* does have high species diversity with 22 species with concordant DNA and morphology. Some lineages require further investigation as their mtDNA was not reciprocally monophyletic with respect to other putative species, while others comprised multiple divergent mtDNA lineages and may represent more that a single species. The relationships among species indicate that New Zealand *Hemiandrus* consists of two mtDNA clades. The explanation of why *Hemiandrus* has high diversity compared to other Anostostomatidae genera is unknown but it may be that *Hemiandrus* represents more than a single genus and this will be explored in the following chapter.

Appendix

Appendix 5.1. Specimens used for phylogenetic analysis.

code	species	location	code	species	location
GW664	ALIUS	Moanui, BP	GW460	*electra*	Springs Junction, BR
GW734	ALIUS	Waikaremoana, GB	FD6	*focalis*	Codfish Island, SI
GW94a	ALIUS	Waikaremoana, GB	GW1069	*focalis*	Awarua Bay, SL
GW834	ALIUS	Awatuna, WD	GW8	*focalis*	Harris Saddle, OL
GW899	ALIUS	Denniston, NN	FD7	*focalis*	Eyre Mountains, OL
GW896A	ALIUS	Denniston, NN	FD8	*superba*	Sinbad Valley, FD
GW896B	ALIUS	Denniston, NN	FD10	*superba*	Sinbad Valley, FD
GW896C	ALIUS	Denniston, NN	FD11	*superba*	Sinbad Valley, FD
GW35a	*subantarcticus*	Snares Islands, SN	FD5	*superba*	Sinbad Valley, FD
GW792	*subantarcticus*	Snares Islands, SN	GW114	“timaru”	Kurinui, DN
GW988	*subantarcticus*	Snares Islands, SN	GW211	“timaru”	Burkes Pass, MK
GW989	*subantarcticus*	Snares Islands, SN	GW1096	“furoviarius”	Tekapo, MK
GW561	“madisylvestris”	Eglinton Valley, FD	GW1097	“furoviarius”	Tekapo, MK
GW566	“madisylvestris”	Eglinton Valley, FD	GW1098	“furoviarius”	Tekapo, MK
FD1	*nitaweta*	Sinbad Valley, FD	GW674	“kapiti”	Kapiti Island, WN
FD3	*nitaweta*	Sinbad Valley, FD	GW682	“kapiti”	Kapiti Island, WN
FD15	*nitaweta*	Sinbad Valley, FD	GW431	*pallitarsis*	Oakura Beach, TK
GW27	PATURAU	Paturau River, NN	GW66	*pallitarsis*	Waikaremoana, GB
GW1026B	NORTH	Mt Richmond Forest Park, NN	NG	*pallitarsis*	Bledisloe Park, WN
GW49A	NORTH	Puketi Forest, ND	GW251	“horomaka”	Kaikoura, KA
GW624	NORTH	Pureora, TO	GW120	“horomaka”	Banks Peninsula, MC
GW919	NORTH	Mt Taranaki, TK	GW127	“horomaka”	Porters Pass, MC
FD4	*maculifrons*	Eglinton Valley, FD	GW129	“horomaka”	Porters Pass, MC
GW909B	*maculifrons*	Arthurs Pass, NC	GW240	*bilobatus*	Wellington, WN
GW1018	*maculifrons*	Lewis Pass, BR	GW241	*bilobatus*	Wellington, WN
GW815	CENTRAL	Kahuterawa,	GW25	*bilobatus*	Wellington, WN

		WN			
GW611	CENTRAL	Tararuas, WN	GW657	*bilobatus*	Mana Island, WN
GW916B	CENTRAL	Ruahines, RI	GW193	“promontorius”	Seaview, KA
GW1013	CENTRAL	Lewis Pass, BR	GW122	“promontorius”	Marfell's Beach, KA
GW62	“elegans”	Moehau, CL	GW55	“promontorius”	Marfell's Beach, KA
GW208	“elegans”	Whareorino Forest, WO	GW586	“promontorius”	Lower Awatere Valley, MB
GW125	*maia*	Otago Penninsula, DN	GW581	“promontorius”	Lower Awatere Valley, MB
GW118	*maia*	Kurinui, DN	GW584	“promontorius”	Lower Awatere Valley, MB
GW136	*maia*	Blue Mountains, SL	GW958	“onokis”	Renwick, MB
GW137	“disparalis”	Gouland Downs, NN	GW491	“onokis”	Middle Clarence Valley, KA
GW216	“disparalis”	Mt Haidinger, NN	GW1031	“onokis”	Mt Richmond Forest Park, NN
GW139	“disparalis”	Nelson Lakes, BR	GW867	“onokis”	Upper Clarence Valley, MB
GW33A	“disparalis”	Nelson Lakes, BR	GW869	“onokis”	Upper Clarence Valley, MB
GW522	“hapuku”	Orr Stream, MB	GW871	“onokis”	Upper Clarence Valley, MB
GW1059	“tapuae o uenuku”	Mt Tapuae-o-Uenuku, KA	GW54	“vicinus”	Whites Bay, SD
GW669	“hapuku”	Kowhai River, KA	GW1037	“vicinus”	Maitai Valley, NN
GW668	“hapuku”	Kowhai River, KA	GW1047	“vicinus”	Cable Bay track, NN
GW101	*electra*	Rameka, NN	GW602	“vicinus”	Te Rua Bay, SD
GW138	*electra*	Nelson Lakes, BR	GW717	“vicinus”	Manaroa, SD
GW96	*electra*	Kahurangi NP, NN	GW1033	“vicinus”	Nelson, NN
GW124	*electra*	Blackball, BR	GW1040	“vicinus”	Nelson, NN
GW1084	*electra*	Te Kuha, NN	GW1043	“vicinus”	Dodson Valley, NN

Appendix 5.2. New locations for ground wētā taxon distributions.

Taxon	New locations
“elegans”	Whareorino Forest (WO), Puketi Forest (ND), Waipoua Forest (ND)
“disparalis”	St Arnaud (BR), Mt Richmond Forest Park (NN), Punakaiki (BR), Denniston Plateau (NN), Kumara Junction (WD)
electra	St Arnaud (BR), Mt Richmond Forest Park (NN), Blackball (BR), Springs Junction (BR)
ALIUS	Hokitika (WD), Denniston Plateau (NN), Springs Junction (BR), St Arnaud (BR)
“vicinus”	Nelson (NN)
pallitarsis	Tolaga Bay (GB), Rocky Hills (WA), Waiwera (AK)
bilobatus	NA
maculifrons	NA
NORTH	NA
CENTRAL	NA
“madisylvestris”	NA
maia	Mt Kyeburn (CO), Awarua Plains (SL)
superba	NA
nitaweta	Takahe Valley (FD)
“timaru”	Rangitata River (SC), Burkes Pass (MK), Mt Hutt (MC)
fiordensis	Paparoa Ranges (BR), Sinbad Gully (FD)
focalis	Codfish Island (SI), Awarua Plains (SL), Longwood Range (SL), Takahe Valley (FD), Wolfe Flat (FD), Mt Doris (WD), Red Mountain (WD), Mt Annetta (WD)
“horomaka”	Porters Pass (MC), Kaikoura (KA)
“onokis”	Mt Richmond Forest Park (NN), Middle and Upper Clarence Valley (KA, MB)
subantarcticus	NA
PATURAU	NA
“saxatilis”	NA
“kapiti”	NA
“hapuku”	Mount Tapuae-o-Uenuku (KA), Goat Valley Stream (KA), Kahutara River(KA), Kowhai River (KA), Mt Fyffe (KA), Orr Stream (MB).

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Chapter 6. New Zealand ground wētā (Anostostomatidae: *Hemiandrus*): descriptions of two species with notes on their biology

Abstract

Although the New Zealand ground wētā (Anostostomatidae: *Hemiandrus*) are widespread and abundant, little has been described of their ecology and behaviour. Within the genus several lineages have evolved with ovipositors that are unusually short for this orthopteran family. Some species with this derived morphological character also exhibit maternal care of eggs and offspring. Two new species are described here, *Hemiandrus maia* sp. nov. and *Hemiandrus electra* sp. nov. Although morphologically similar with medium length ovipositors, they are not sister taxa and live at opposite ends of South Island, New Zealand. The behaviour of *Hemiandrus maia* sp. nov. was studied using burrow door re-construction as a key to activity patterns. Observations at night and burrow excavation during the day were used to identify features of their behaviour. Maternal care of both eggs and nymphs was observed. *Hemiandrus maia* sp. nov. were shown to eat fruit, invertebrates and seeds without discrimination.

Taylor Smith, B. L., Morgan-Richards, M., & Trewick, S. A. (2013). New Zealand ground wētā (Anostostomatidae: *Hemiandrus*): descriptions of two species with notes on their biology. *New Zealand Journal of Zoology, 40*(4), 314-329. doi: 10.1080/03014223.2013.804422

Introduction

Wētā (Insecta: Orthoptera: Anostostomatidae) are an important and prominent group in New Zealand ecology (Brockie 1992). The family in New Zealand comprises three main lineages, tree (*Hemideina* White, 1846) and giant (*Deinacrida* White, 1842) wētā, tusked wētā (*Anisoura* Ander, 1938, *Motuweta* Johns, 1997) and ground wētā (*Hemiandrus* Ander, 1938) (Johns 1997; Trewick & Morgan-Richards 2004, 2005). Together, they comprise some 60 species that occupy diverse habitats from lowland forest to the alpine zone. Among them, the ground wētā are the most speciose but also the most poorly characterized and in need of most taxonomic and ecological work. Johns (1997) estimated that there might be as many as 40 undescribed species but there are nine described to date (Johns 1997; Johns 2001; Jewell 2007). In addition an estimated 10 undescribed species of *Hemiandrus* are endemic to Australia (Johns 1997), although molecular data indicate that these may not be monophyletic with the New Zealand *Hemiandrus* (Pratt *et al.* 2008).

New Zealand *Hemiandrus* ground wētā are found in lowland forest, riverbeds, alpine herb fields and suburban gardens but all share the characteristic of occupying holes in the ground as daytime refuges. They emerge at night to forage and mate, usually sealing the entrance of their burrow during the day with a soil plug. Although some are relatively large (30 mm) and abundant (e.g. Brockie 1992), daytime concealment below ground and nocturnal activity render ground wētā difficult to observe. This probably explains why so little is known of *Hemiandrus* ecology. All species of ground wētā are nocturnal and some, perhaps all, are predominantly predatory (Cary 1983). Many species climb trees at night but others are more commonly encountered on the ground and on low vegetation (Brockie 1992; Wahid 1978; BL Taylor Smith, M Morgan-Richards, SA Trewick pers. obs.). Some ecological research has been undertaken on *Hemiandrus maculifrons* (Walker, 1869) (Cary 1983), *Hemiandrus subantarcticus* (Salmon, 1950) (Butts 1983), *Hemiandrus* “peninsularis”/”horomaka” (Wahid 1978) and *Hemiandrus pallitarsis* (Walker, 1869) (Chappell *et al.* 2012; Gwynne 2002; Gwynne 2004, 2005).

Most Anostostomatidae females lay eggs into the soil using a long slender ovipositor; however, ovipositor length differs markedly among species of New Zealand *Hemiandrus*. Some species have a very small ovipositor that is scarcely visible. The species lacking an obvious ovipositor were at one time taxonomically distinguished as *Hemiandrus* from *Zealandosandrus* (Salmon, 1950) with fully developed ovipositors, but later synonymized (Johns 1997, 2001). A long ovipositor is common in *Hemiandrus* and other members of the family and can be considered the ancestral condition. Reduction in ovipositor length has therefore been inferred as having occurred at least three times in the New Zealand *Hemiandrus* (Pratt *et al.* 2008). Of the seven putative species with extremely short ovipositors (Johns 2001), six are narrow endemics (Chappell *et al.* 2012). Two species, *Hemiandrus maia* sp. nov. and *Hemiandrus electra* sp. nov. are very similar in appearance, but we can infer from molecular phylogenetic evidence that their medium length ovipositors are independently derived (Pratt *et al.* 2008).

Females of the widespread North Island species *Hemiandrus pallitarsis*, which lacks an obvious ovipositor, deposit about 50 eggs in their own burrows, where females remain with eggs and nymphs during winter and spring (Gwynne 2004; SA Trewick pers. obs.). Adult female *H. pallitarsis* are unusual in that they possess a distinctive forked structure on the sixth abdominal sternite (Gwynne 2002) that is involved in transfer of a nuptial gift during mating (Gwynne 2005). In species with long ovipositors, such as *Hemiandrus focalis* (Hutton, 1897), and in other anostostomatids (e.g. Angulo 2001; Stringer 2001), females deposit eggs into soil or moss when active outside their burrows (M Morgan-Richards pers. obs.), and there is no evidence that they have any further involvement with their offspring.

Materials and methods

Taxonomic methods

All specimens were preserved in 95% ethanol and are part of the Phoenix Lab insect collection at Massey University, Palmerston North. All ground wētā in this collection have a unique accession number with the prefix GW. Specimens were examined using an Olympus SZX7 Zoom Stereomicroscope with an attached SC100 digital camera. Body length (length from frons to distal margin of ninth abdominal tergite), head width (width of widest part of head), pronotum length (width of widest part of pronotum) and femur length measurements were made using digital callipers. Photographs of male terminalia were captured using the Olympus Image Analysis Software, which was also used to measure ovipositor length and pronotum width. Images of whole specimens were captured using a Canon EOS 40D digital camera.

Abbreviations

GW, ground wētā in the Phoenix Lab collection (Massey University, Palmerston North); MONZ, Museum of New Zealand Te Papa Tongarewa; BL, body length is the length of the insect excluding the ovipositor and other appendages; MP3, third segment of the maxillary palps; MP4, fourth segment of the maxillary palps; MP5, fifth segment of the maxillary palps; T1, first abdominal tergite; T2, second abdominal tergite; T3, third abdominal tergite; T8, eighth abdominal tergite; T9, ninth abdominal tergite; T10, tenth abdominal tergite. Area codes for recording specimen localities (Crosby *et al.* 1976): NN, Nelson; BR, Buller; DN, Dunedin; SL, Southland.

Activity patterns

Detailed observations of *H. maia* sp. nov. were made on a large (750 ha) private land block in the Kurinui Creek catchment, North Otago, New Zealand. The study site is at 482 m above sea level, 45°21′56.2″S, 170°43′02.2″E. At Kurinui, *H. maia* sp. nov. is sympatric with a long-ovipositor ground wētā, *Hemiandrus* "timaru" (Johns 2001), and both are abundant. Here, we report on observations carried out 24–27 December 2007, 31 December 2009 to 01 January 2010 and 22–23 January 2013.

Night observations were made immediately after dark (22.30 h) in summer. Investigators cautiously searched open ground, forest floor and vegetation to head height by torchlight. Ground wētā observed were captured by hand, sexed and identified, see species description below.

During the day, the entrances of 75 ground wētā burrows were identified on a north-facing cutting and their soil doors were lightly marked with quick-drying white aerosol paint (Fig 6.1). Each burrow door was uniquely identified. On the following day and on three subsequent days the door of each burrow was examined. Wētā leave their burrow during the night by breaking up the soil plug at the burrow entrance, and on returning to their burrow they rebuild the door using the surrounding soil. Newly constructed doors were therefore clearly visible as brown against the surrounding white paint (see Figs 6.1B, 6.1C). If the door had been reconstructed overnight then this was recorded and the burrow entrance again sprayed with paint. After 3 days of observation, nine of the monitored burrows were excavated, along with a further 11 unmonitored burrows.

Food choice experiment

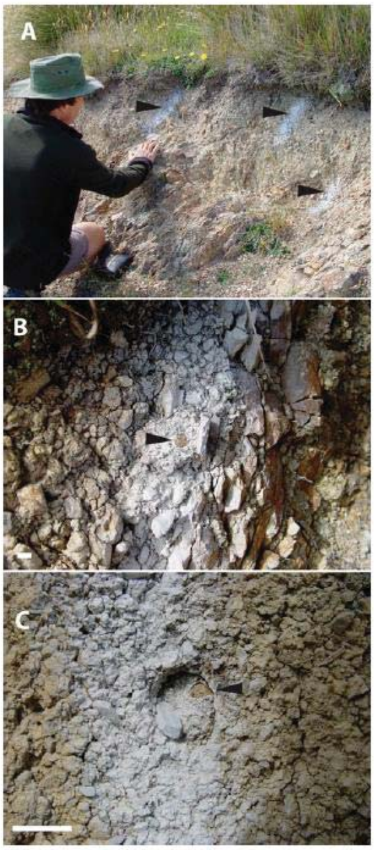
Eighteen food choice arenas were set up at dusk (21.45 h) at the base of a different earth bank in which *Hemiandrus* had made burrows (Fig 6.2A). Each arena was separated by about 2 m on a transect following the base of the earth bank. This spacing was appropriate for the density of wētā indicated by observations made previously. Each 60×60-mm arena was provisioned with three food items held in place with metal pins: 5 mm^3 fresh apricot; whole *Calliphora* sp. fly (previously killed by freezing); 8–12 grains of oatmeal. Apricot was used because this fruit is known to be eaten by wild *Hemiandrus* in orchards (Wahid 1978). *Hemiandrus* have been observed feeding on Diptera in the field so flies were caught on location, killed by freezing and then thawed before placement. Oatmeal was chosen as a surrogate for seed-like plant carbohydrate potentially available to ground wētā and known to be attractive to them (Johns 2001). The food items were arranged as points of a triangle within each arena. Observations were made between 23.00 h and 00.15 h following provisioning. Arenas were checked repeatedly, but each individual animal observed eating was recorded once only to ensure independence, and only when all three foods were available.

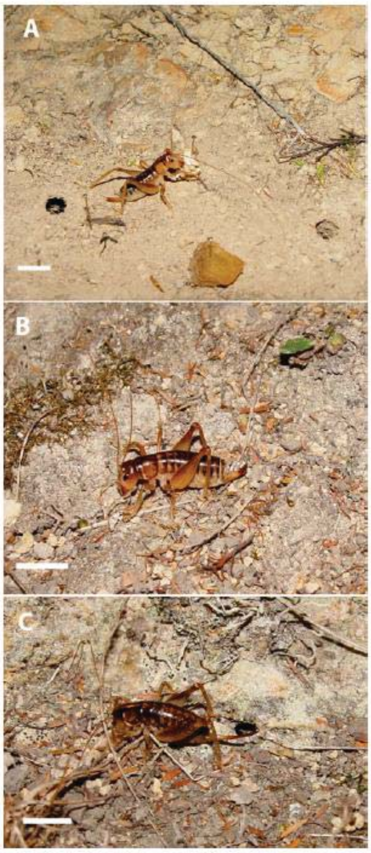
Population size estimation

We marked all the ground wētā we could see in a specified area at a particular time. We did this for *H. maia* sp. nov. at Kurinui within an area of approximately 400 m^2, and for *H. electra* sp. nov. at Awaroa (Golden Bay, Tasman District) within an area of approximately 380 m^2. The following night, we counted all the ground wētā in the same area, at the same time, and recorded how many had been marked from the previous night. We estimated population size using the Lincoln–Peterson estimator (Lincoln 1930; Petersen 1896) with the Chapman modification, which reduces the bias associated with small population sizes (Chapman 1951), using the following equation:

$$N = \frac{(M+1)(C+1)}{R+1} - 1$$

where N=Estimate of total population size, M=Total number of animals captured and marked on the first visit, C=Total number of animals captured on the second visit and R=Number of animals captured on the first visit that were then recaptured on the second visit.





Left: Figure 6.1. Burrow door reconstruction used to monitor the activity of the New Zealand ground wētā, *Hemiandrus maia* sp. nov. The entrances of burrows were identified and their soil doors were lightly sprayed with white paint. On subsequent days the door of each burrow was examined. Wētā leave their burrow during the night by breaking up the soil plug at the burrow entrance, and on returning to their burrow they rebuild the door using the surrounding soil. **A**, Cutting with three marked burrow entrances of the ground wētā *H. maia* sp. nov. at Kurinui, North Otago indicated by black arrows. **B**, The soil plug (burrow door) after reconstruction is visible against the white paint. **C**, Small circular reconstructions within the burrow door reveal activity of ground wētā nymphs. Scale bar = 10 mm.

Right: Figure 6.2. Food choice arenas were set up at the base of an earth bank, the habitat of New Zealand ground wētā, *Hemiandrus maia* sp. nov., at Kurinui, North Otago. Also pictured is a female of the sympatric *Hemiandrus* "timaru", which is easily distinguished from *H. maia* sp. nov. by its long ovipositor. **A**, Food-choice arena with ground wētā feeding on oats, fly already consumed. **B**, Adult female *H. maia* sp. nov. **C**, Adult female *H.* "timaru". Scale bar = 10 mm.

Species description: *Hemiandrus maia* sp. nov.

A combination of morphological structures provided useful diagnostic characters for *Hemiandrus* (Fig 6.3).

Class: **Insecta**
Order: **Orthoptera**
Suborder: **Ensifera**
Superfamily: **Tettigoniodea**
Family: **Anostostomatidae** Saussure, 1859
Genus: ***Hemiandrus*** Ander, 1938.
Type species: *Hemiandrus furcifer* Ander, 1938

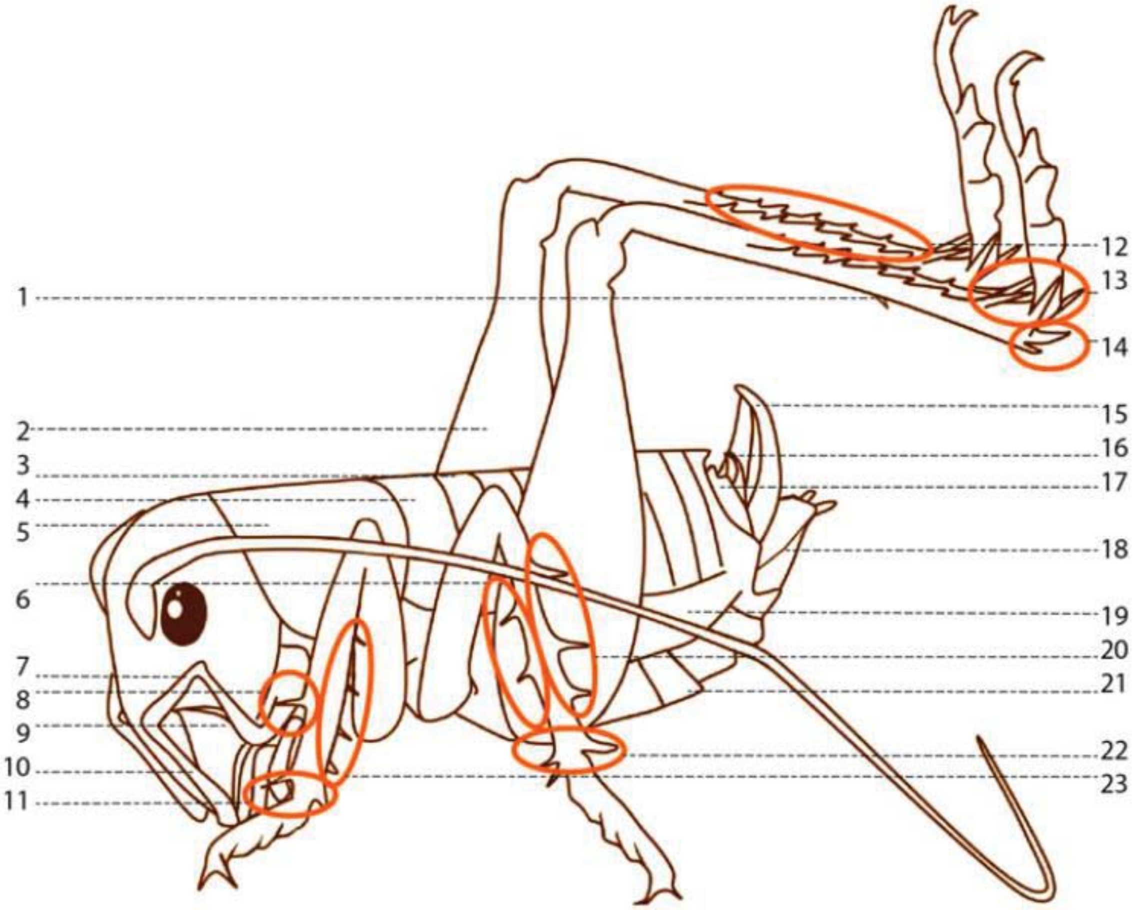


Figure 6.3. External morphological characters useful in the identification of *Hemiandrus* species. 1. Hind tibia: inferior articulated spines. 2. Hind femur: retrolateral surface. 3. Metanotum. 4. Mesonotum. 5. Pronotum. 6. Mid tibia: superior prolateral spines. 7. MP4. 8. Fore tibia: superior prolateral spine. 9. MP3. 10. MP5. 11. Fore tibia: apical spines. 12. Hind tibia: superior fixed spines. 13. Hind tibia: subapical spines. 14. Hind tibia: apical spines. 15. Cerci. 16. Paranal processes. 17. T10 with falci. 18. Subgenital plate. 19. Pleural membrane. 20. Mid tibia: retrolateral spines. 21. Abdominal sternites. 22. Mid tibia: apical spines. 23. Fore tibia: inferior retrolateral spines.

Diagnosis:

A medium-sized ground wētā found in Otago (DN, SL), New Zealand, with the following traits: MP3 bare, MP4 55% pilose, 11 to 17 antennomeres. Body colour when live is orange-brown, pronota with large lateral pale patches; fore tibiae with a single superior prolateral spine (excluding apical spine); mid tibiae with two superior prolateral spines and three superior retrolateral spines (excluding apical spines). Females have moderately short ovipositors and maternal care. Most similar to *H. electra* sp. nov., which is found in northern South Island and distinguished by male terminalia, number of tergal stridulatory pegs and body proportions (Table 6.1, Fig 6.4).

Hemiandrus maia sp. nov. is sympatric in parts of its distribution with *H.* "timaru", *H. focalis* and *H. maculifrons*, but is distinguishable by body size, ovipositor length, the number of mid tibial spines and MP3 pilosity (Table 6.2).

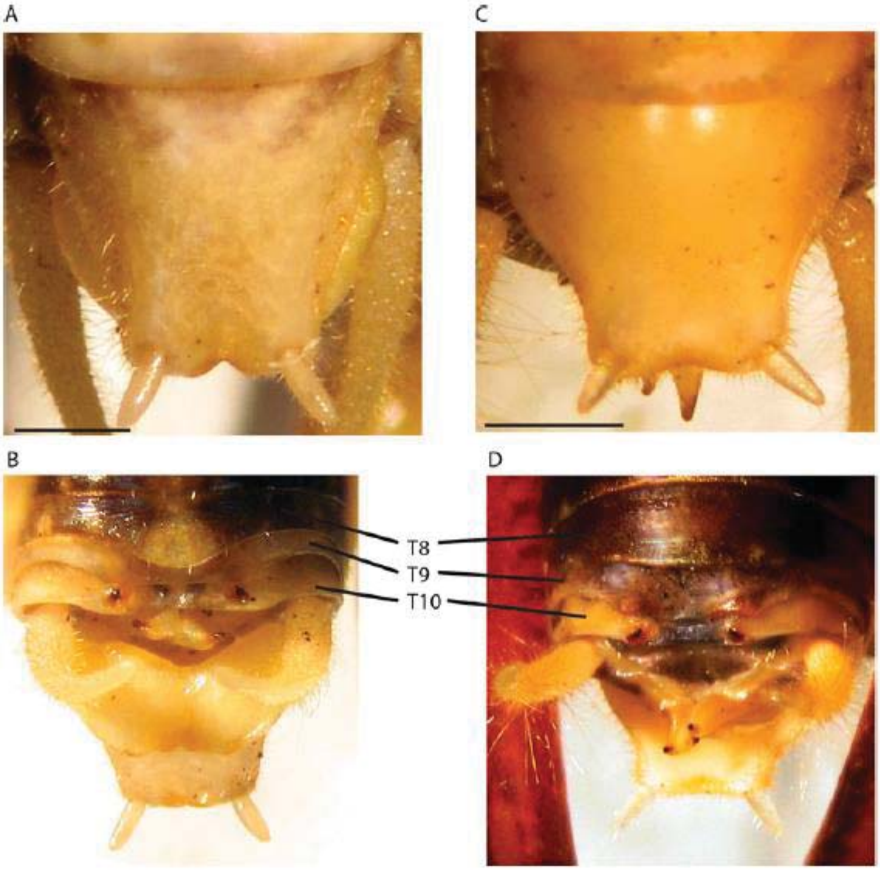


Figure 6.4. *Hemiandrus maia* sp. nov. adult male. **A.** Subgenital plate (ventral view). **B.** Male terminalia (dorsal view). *Hemiandrus electra* sp. nov. adult male. **C.** Subgenital plate (ventral view). **D.** Male terminalia (dorsal view). T8-10 indicates abdominal tergites. Scale bar = 1 mm.

Table 6.1. Morphological characters that distinguish the New Zealand ground wētā *Hemiandrus maia* sp. nov. and *Hemiandrus electra* sp. nov.

Character	*H. maia* sp. nov.	*H. electra* sp. nov.
Length of hind femora relative to head width	2.3–2.7 times longer	2.7–3.1 times longer
Number of stridulatory pegs on T1-T3	T1: 50–70 T2: 60–80 T3: 30–60	T1: 90–120 T2: 130–170 T3:100–150
Male terminalia:		
Subgenital plate and T8-T10		
Subgenital plate	Notched posterior margin	Simple or very slightly concave posterior margin
T8	Median lobe	Simple
T9	Flattened lobe	Weakly bilobed
T10	Pale between falci	Grey between falci

Table 6.2. Morphological characters that distinguish the New Zealand ground wētā *Hemiandrus maia* sp. nov. from other *Hemiandrus* found within its range. Mid tibial superior retrolateral spines excludes apical spines unlike Johns (2001).

Character	*H. maia* sp. nov.	*H.* 'timaru'	*H. focalis*	*H. maculifrons*
Body size	Medium	Small	Large	Small
(Adult head width as proxy)	4.9–6.9 mm	4.9 mm	6.8–9.6 mm	3.7–4.9 mm
	$n = 23$	$n = 2$	$n = 4$	$n = 67$
Ovipositor length	Moderately short	Long	Long	Long
Mid-tibial superior retrolateral spines	3	2	3	3
MP3 pilosity	None	None	None	Approx. 33%

Etymology:

From the Greek name Maia, the eldest of the Pleiades (in Greek mythology the seven daughters of Pleione and Atlas), meaning 'mother' or 'good mother'. This name is given to this species to reflect the maternal care exhibited. *Hemiandrus maia* is probably the tag-named entity *Hemiandrus* "evansae" (Johns 2001).

Description:

Adult BL: males 16.8–24.7 mm (n=13), females 19.7–25.7 mm (n = 10) (Fig 6.5).

Head. Head shiny, darker on top; labrum cream, setose; mandibles brown; scape, pedicel and antennomeres cream and light brown; antennae longer than body; flagella composed of 11–17 antennomeres; eyes black; maxillary palps cream; MP5 100% pilose, MP4 55% pilose, MP3 bare.

Thorax. Pronotum wider than long. Pronotum, mesonotum and metanotum dark brown with large pale patches either side of a narrow pale dorsal mid-line. Sternum cream and/or light brown.

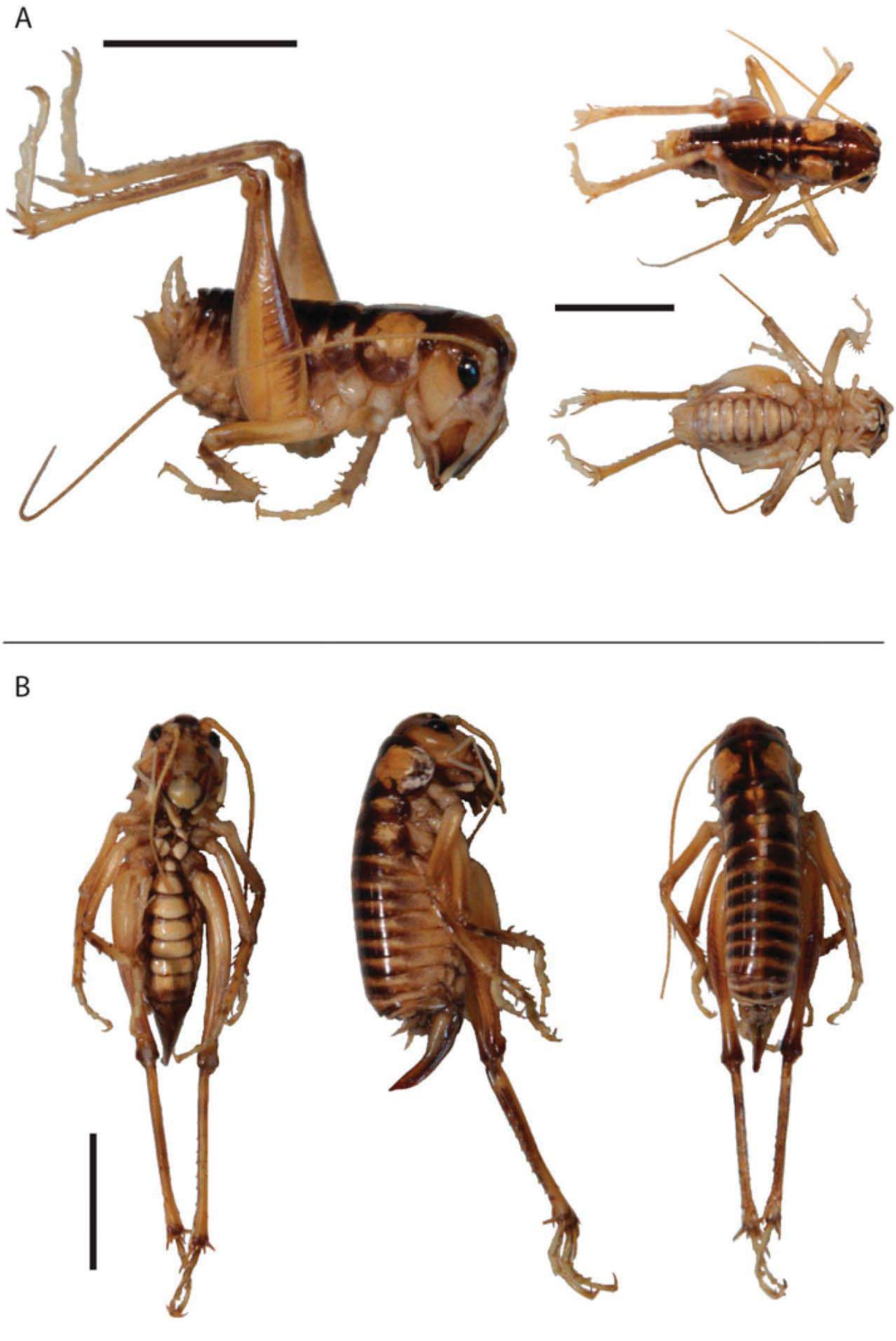


Figure 6.5. *Hemiandrus maia* sp. nov. **A.** Adult male. **B.** Adult female. Scale bar = 10 mm.

Legs. Moderately long (hind femora 2.3 to 2.7 times head width); coxae and trochanters cream and/or brown; femora and tibiae cream with brown patches. Fore tibiae with cream articulated spines with brown tips arranged in asymmetrical form as follows: four apical spines; one spine positioned medially on superior prolateral angle; none on superior retrolateral angle; four along inferior prolateral angle; four along inferior retrolateral angle. Mid tibiae with cream articulated spines with brown tips arranged asymmetrically as follows: four apical spines; two spines spaced along superior prolateral angle; three spines spaced along superior retrolateral angle; four spines spaced along inferior retrolateral angle; four spines spaced along inferior prolateral angle. Hind tibiae with articulated cream spines with brown tips arranged as follows: four apicals; two very large sub-apical spines on each of the superior prolateral and retrolateral angles. Seven to nine fixed spines spaced along the superior prolateral and retrolateral angles. Two to four very small articulated spines positioned along the inferior prolateral angle and zero to two on the inferior retrolateral angle. Hind femora with a patch of 5–40 stridulatory pegs on the retrolateral surface. Tarsi cream, setose, four-segmented, first segment with foot-pad divided into two, other segments with a single foot-pad.

Abdomen. Shiny brown with a pale dorsal midline and pale patches either side of the abdomen; each tergite lighter anteriorly, darker posteriorly, sometimes giving a banded effect; sides of the abdomen dull and pilose; pleural membrane brown or cream and pilose; sternites cream but darker and more pilose towards lateral and anterior edges; T1 with 50–70 stridulatory pegs on each side; T2 with 60–80 stridulatory pegs on each side; T3 with 30–60 stridulatory pegs on each side; fourth and fifth tergites with fewer pegs; cerci cream.

Males. Cerci blunt, setose; eighth abdominal tergite T8 margin medially lobed; ninth abdominal tergite T9 margin with a flattened lobe; T10 between falci pale; subgenital plate with notched posterior margin; paranal processes long, pointed with darkened tip (Fig 6.4).

Females. T9 simple; paired pits on sixth sternite; cerci long, pointed, bare tip; ovipositor moderately short: 6.5–8.6 mm (n = 8).

Distribution:

Southeast South Island New Zealand; Otago and Southland (DN, SL) (Fig 6.6A). They have been collected from the following localities: Kurinui Creek catchment, North Otago (DN) (45°21′56.2″S, 170°43′02.2″E); Caversham, Dunedin (DN) (45°53′54.5″S, 170°28′34.6″E); Portobello, Otago (DN) (45°50′22.3″S, 170°39′07.6″E); Blue Mountains (SL) (45°56′19.5″S, 169°21′13.2″E).

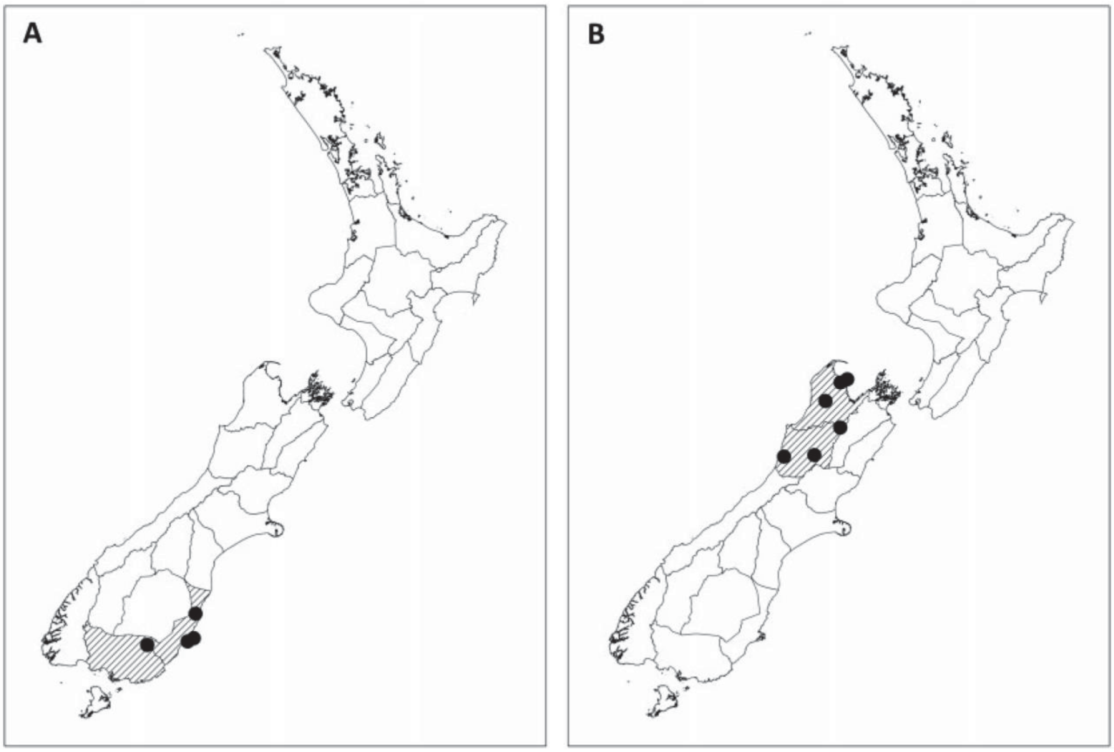


Figure 6.6. The distributions of two New Zealand ground wētā, showing the Crosby et al. (1976) regions in which they have been recorded. Spots indicate position of sites reported in the descriptions. **A**. *Hemiandrus maia* sp. nov. is known from southeast South Island (DN, SL). **B**. *Hemiandrus electra* sp. nov. from northwest South Island (NN, BR).

Type data:

Holotype: Adult male (Fig 6.5A): 17.1 mm collected January 2010 from Kurinui (DN) (45°21′56.2″S, 170°43′02.2″E) Hampden, North Otago, SA Trewick and M Morgan-Richards (MONZ AI.031569; GW 764). Paratype adult female (Fig 6.5B): 22.6 mm, collected January 2006 from Kurinui (DN) (45°21′56.2″S, 170°43′02.2″E) Hampden, North Otago, SA Trewick and M Morgan-Richards (MONZ AI.031570; GW115).

Additional material examined:

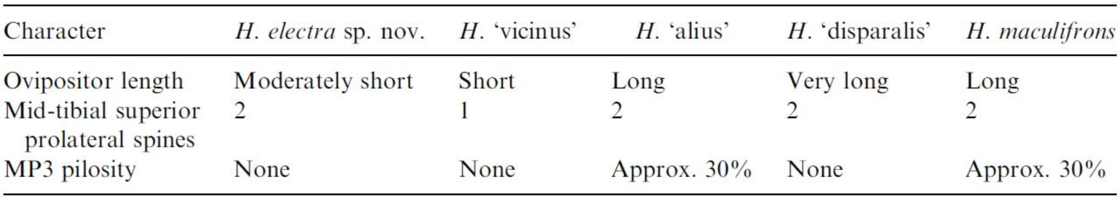
Caversham, Dunedin (DN) (GW 06A; 6B; 11; 37A; 37B; 38B); Portobello, Dunedin (DN) (GW 125, GenBank accession EU676744); Blue Mountains (SL) (GW 135; 136, GenBank accession EU676780); Kurinui, Hampden, North Otago (DN) (GW 116; 117; 118, GenBank accession EU676795; GW264; 265; 266; 405; 406; 407; 408; 503; 504; 505; 519; 520; 521; 619). These specimens are held in the Phoenix collection, Massey University.

Species description: *Hemiandrus electra* sp. nov.

Diagnosis:

A medium-sized ground wētā found in Nelson, Marlborough, Tasman and West Coast (NN, BR), New Zealand, with the following traits: MP3 bare, MP4 55% pilose, 13 to 18 antennomeres. Body colour when live is red-brown, pronota with large lateral pale patches; fore tibiae with a single superior prolateral spine (excluding apical spine); mid tibiae with two superior prolateral spines and three superior retrolateral spines (excluding apical spines). Females have moderately short ovipositors. Most similar to *H. maia* sp. nov. and distinguished by number of tergal stridulatory pegs, male terminalia and body proportions (Table 6.1). *Hemiandrus electra* sp. nov. is sympatric in parts of its distribution with *Hemiandrus* "alius", *Hemiandrus* "disparalis", *H. maculifrons*, and possibly *Hemiandrus* "vicinus". Hemiandrus *electra* sp. nov. can be distinguished from these species by ovipositor length, the mid tibial spines and MP3 pilosity (Table 6.3).

Table 6.3. Morphological characters that distinguish the New Zealand ground wētā *Hemiandrus electra* sp. nov. from other *Hemiandrus* found within its range. Mid tibial superior prolateral spines excludes apical spines unlike Johns (2001).



Character	*H. electra* sp. nov.	*H.* 'vicinus'	*H.* 'alius'	*H.* 'disparalis'	*H. maculifrons*
Ovipositor length	Moderately short	Short	Long	Very long	Long
Mid-tibial superior prolateral spines	2	1	2	2	2
MP3 pilosity	None	None	Approx. 30%	None	Approx. 30%

Etymology:

From the Greek name Elektra, in Greek mythology one of the seven Pleiades (daughters of Pleione and Atlas). Elektra is sister to Maia, a reference to the morphological similarity of *H. electra* sp. nov. and *H. maia* sp. nov. The name Elektra is derived from elektron meaning 'amber' and related to elektros meaning 'shining, bright', and is applied here in reference to the bright orange colour of this species. *Hemiandrus electra* sp. nov. is probably the tag-named entity *Hemiandrus* "okiwi" (Johns 2001).

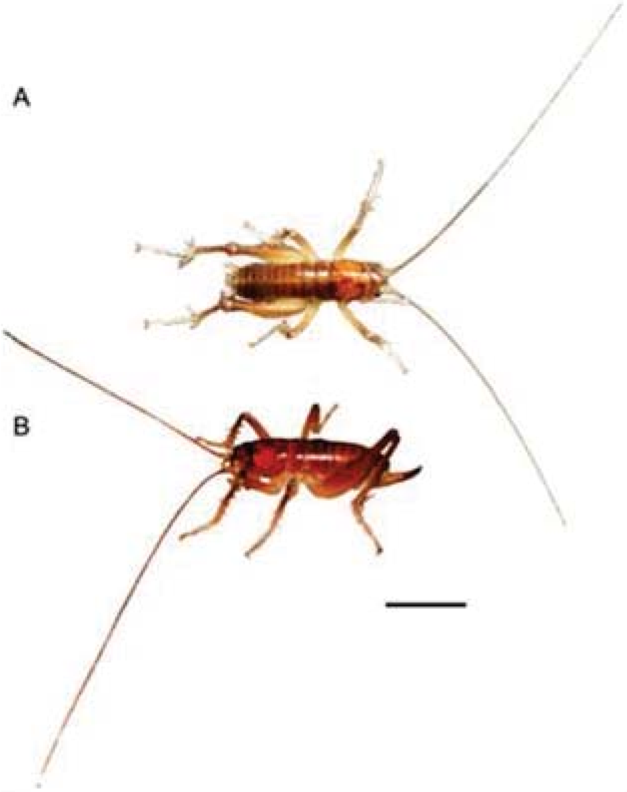


Figure 6.7. *Hemiandrus electra* sp. nov. A, Adult male. B, Adult female. Scale bar = 10 mm.

Description:

Adult BL: males 14.9–21.4 mm (*n*=14), females 18.4–24.1 mm (*n*=7) (Fig 6.7).

Head. Head shiny, darker on top; labrum cream, setose; mandibles brown; scape, pedicel and antennomeres cream and light brown; antennae longer than body; flagella composed of 13–18 antennomeres; eyes black; maxillary palps cream; MP5 100% pilose, MP4 55% pilose, MP3 bare.

Thorax. Pronotum wider than long. Pronotum, mesonotum and metanotum dark brown with large pale patches either side of a narrow pale dorsal mid-line. Sternum cream and/or light brown.

Legs. Long (hind femora 2.7 to 3.1 times head width); coxae and trochanters cream and/or brown; femora and tibiae cream with brown patches. Fore tibiae with cream articulated spines with brown tips arranged in asymmetrical form as follows: four apical spines; one spine positioned medially on superior prolateral angle; none on superior retrolateral angle; four along inferior prolateral angle; four along inferior retrolateral angle. Mid tibiae with cream articulated spines with brown tips arranged asymmetrically as follows: four apical spines; two spines spaced along superior prolateral angle; three spines spaced along superior retrolateral angle; four spines spaced along inferior retrolateral angle; four spines spaced along inferior prolateral angle. Hind tibiae with articulated cream spines with brown tips arranged as follows: four apical spines; two very large sub-apical spines on the superior prolateral and retrolateral angles. Seven to nine fixed spines spaced along the superior prolateral and retrolateral angles. Two to four very small articulated spines positioned along the inferior prolateral angle and zero or one on the inferior retrolateral angle. Hind femora with a patch of 40–70 stridulatory pegs on the retrolateral surface. Tarsi cream, setose, four-segmented, first segment with foot-pad divided into two, other segments with a single foot-pad.

Abdomen. Shiny orange-brown with a pale dorsal midline; pleural membrane brown or cream and pilose; sternites cream but darker and more pilose towards lateral and anterior edges; first tergite with 90–120 stridulatory pegs on each side; T2 with 130–170 stridulatory pegs on each side; T3 with 100–150 stridulatory pegs on each side; fourth and fifth tergites with fewer stridulatory pegs; cerci cream.

Males. Cerci blunt, setose; T8 margin simple; T9 margin weakly bilobed; T10 area between falci grey; subgenital plate posterior margin simple to slightly concave; paranal processes long, pointed with darkened tip (Fig 6.4).

Females. T9 simple; paired pits on sixth sternite; cerci long, pointed, bare tip; ovipositor moderately short. 6.1–6.7 mm (*n* = 4).

Distribution:

Northwest South Island; Nelson, Marlborough, Tasman and West Coast (NN, BR), New Zealand (Fig 6.6B). They have been collected from the following localities: Awaroa, Tasman (NN) (40°51′54.1″S, 173°01′55.4″E); St Arnaud, Tasman (BR) (41°48′06.1″S, 172°51′00.6″E); Kahurangi National Park, Tasman (NN) (41°17′08.2″S, 172°28′16.8″E); Rameka, Tasman (NN) (40°55′35.5″S, 172°51′27.3″E); Blackball, West Coast (BR) (42°21′07.7″S, 171°22′43.5″E); Spring's Junction, West Coast (BR) (42°19′54.6″S, 172°10′20.9″E).

Type data:

Holotype: Adult male (Fig 6.7A): 19.3 mm, collected January 2012 from Awaroa, Tasman (NN) (40°51′54.1″S, 173°01′55.4″E), SA Trewick and M Morgan-Richards (MONZ AI.031571; GW719). Paratype adult female (Fig 6.7B): 22.9 mm, collected February 2005 from Awaroa, Tasman (NN) (40°51′54.1″S, 173°01′55.4″E), SA Trewick and M Morgan-Richards (MONZ AI.031572; GW86A).

Additional material examined:

Awaroa, Tasman (NN) (GW 67; 86B; 476; 477; 589; 718; 720; 721; 722; 723; 724; 791; 795); St Arnaud, Tasman (BR) (GW 43; 138, GenBank accession EU676783; GW 140; 482); Kahurangi National Park, Tasman (NN) (GW 96); Rameka, Tasman (NN) (GW 101, GenBank accession EU676741; GW 103; 107; 579; 580); Blackball, West Coast (BR) (GW 124); Spring's Junction, West Coast (BR) (GW 460). These specimens are held in the Phoenix collection, Massey University.

Results and discussion

Night observations at Kurinui, Otago

Hemiandrus maia sp. nov. (Fig 6.2B) were observed in the field to feed on naturally available invertebrates (including moths, flies, bees and freshly emerged antlions). All ground wētā were observed on the ground or on vegetation < 50 cm from the ground. Two *Hemiandrus* species were observed at night, in the same area, however, no burrows of the long-ovipositor ground wētā (*H.* "timaru") were identified (Fig 6.2C). At dusk, some ground wētā removed their burrow doors and sat at the entrance. Nymphs of *H. maia* sp. nov. left burrows via small holes made within larger burrow doors (Fig 6.1C).

Activity

Seventy-five ground wētā burrows were marked and observations were made of door reconstructions over 3 days, involving 225 burrow-nights in total. Sixty-six percent of all burrows showed evidence of wētā activity during one, two or three nights (Fig 6.8A). Most burrow entrances were closed with a circle of new soil approximately 10 mm in diameter (Fig 6.1B). On two occasions (from n=225 observations) burrow entrances were not resealed during the day. This comprised one small hole, and one normal-sized entrance that was subsequently closed.

We observed that some burrows had only a small circular section (1–2 mm in diameter) of their doors reconstructed (Fig 6.1C) and these burrows were scored as 'not active' as a full-grown wētā would require a larger hole for exiting the burrow. However, night observations showed that in some doors of normal size (approximately 10-mm diameter) a small hole was made and wētā nymphs left the burrow by this means. In our set of 75 marked burrows, nine had small holes made and filled during one or two nights, and three of these also had the full door reconstructed on other nights (Fig 6.8A).

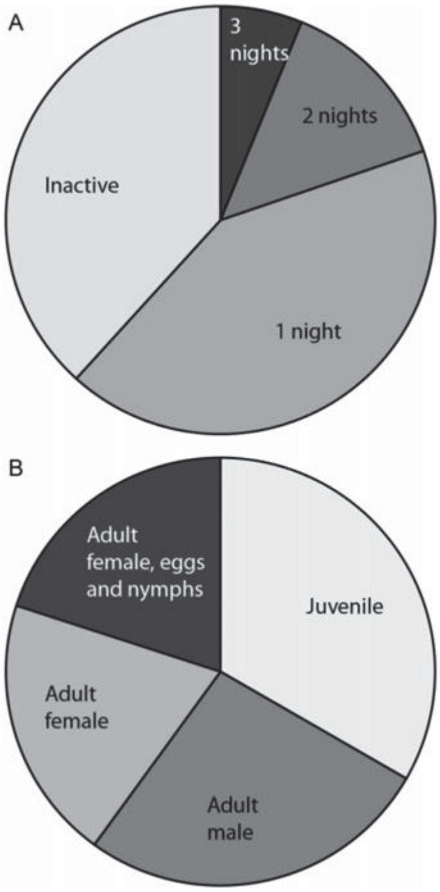


Figure 6.8. Activity and occupancy of burrows by the ground wētā *Hemiandrus maia* sp. nov. A, The proportion of wētā that were active each night as measured by burrow door reconstruction over three nights (*n* = 75 burrows), six of the burrows recorded as inactive showed evidence that ground wētā nymphs had emerged. B, Occupancy of burrows determined by excavation of sealed burrows (*n* = 15).

A third of all burrows that we marked remained inactive over all three nights. Five burrows (6.7%) were open and closed on each of the three sequential nights, 11 burrows (14.7%) were open and closed on two of the three nights and 34 burrows (45%) were opened and closed once during the three nights. Each night, between 19 (25%) and 31 (41%) of the burrows showed sign of wētā activity. These ground wētā were therefore, on average, active once every 3.17 nights during our observations.

An additional observation night was made following excavation of nine burrows and so represents a smaller sample size. The supplemented data provided these statistics: 76% of burrows showed evidence of wētā activity during one, two or three nights (never four); 24% of 67 burrows remained inactive over all four nights. Eight burrows (12%) were open and closed on three nights, 18 burrows (27%) were active on two of the four nights and 25 burrows (37%) were active once during the four nights. Each night between 17 (25%) and 29 (43%) burrows showed sign of wētā activity. These expanded data provide a similar estimate that these ground wētā were, on average, active once every 3.15 nights.

Burrow occupancy

Twenty burrows were excavated. Burrows were about 8–12 mm wide, and ranged between 25 and 205 mm in length. Most extended horizontally into the bank, but others curved gently downwards for 50–100 mm. Many open burrows that we observed were probably made or used by ground wētā, but were now unoccupied and lacked soil plugs (doors). We found dead ground wētā in two burrows without doors and three doorless burrows were empty. All 15 sealed burrows that we opened contained live ground wētā (Fig 6.8B); however, four of these also had the remains of a dead ground wētā (*H. maia* sp. nov). Six burrows that showed no sign of wētā activity over three nights each contained a live ground wētā; three juveniles (two male, one unknown sex), one adult male, one adult female with eggs, one adult female with nymphs. Of the two excavated burrows that had been active twice in three nights, one contained a single adult male and the other a single adult female. One burrow, home to an adult male, had a new door on all three nights.

Of the 15 excavated burrows containing wētā (nine monitored, plus six not monitored) six contained adult females (Fig 6.8B), and half these wētā had eggs and/or nymphs with them. One adult female was observed inside her burrow with 12 wētā nymphs (see Fig 6.9). There were empty egg shells on the soil of the walls and ceiling of the burrow. On initial observation nymphs and adult all faced the burrow entrance. All excavated burrows contained the species *H. maia* sp. nov.

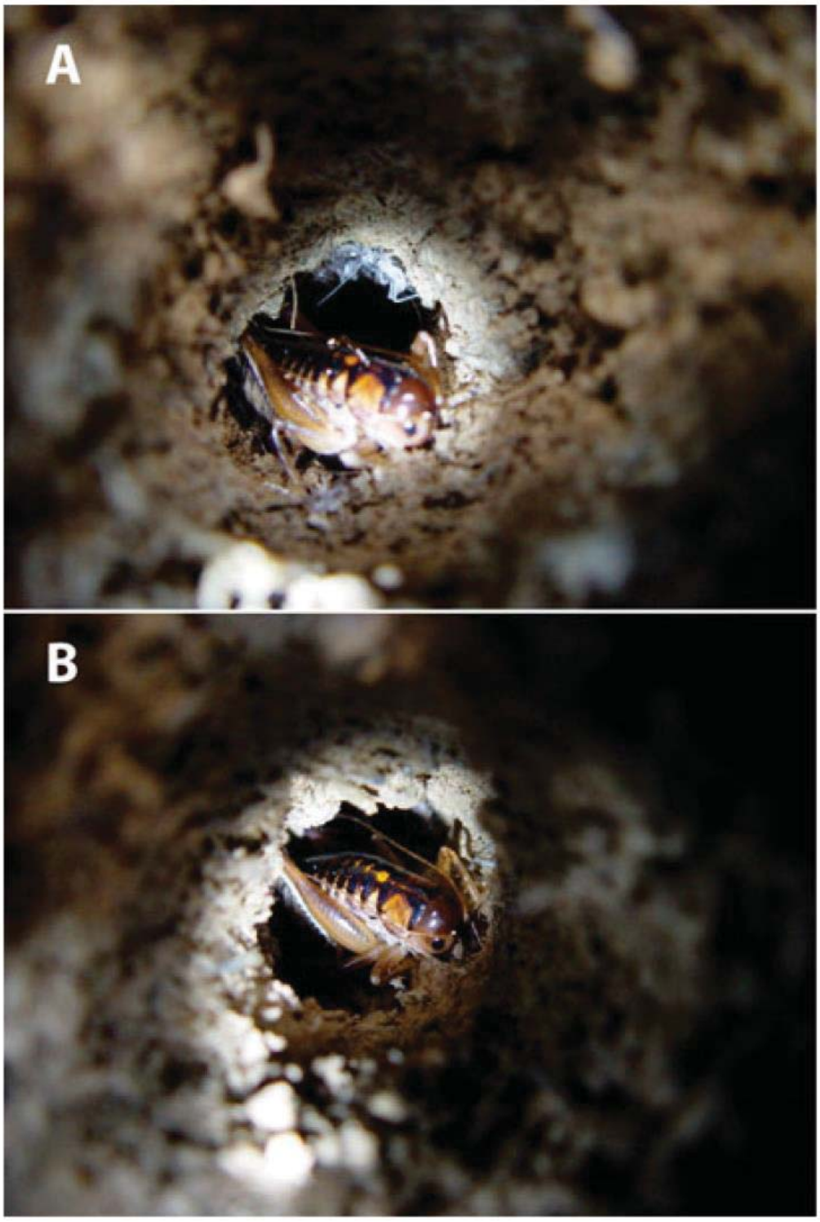


Figure 6.9. Female ground wētā *Hemiandrus maia* sp. nov. 'the good mother', exhibits maternal care of eggs and nymphs.

Table 6.4. Nocturnal insect feeding observations at food-choice arenas at Kurinui, North Otago, New Zealand. Records are independent and restricted to situations where all three foods were available.

	Oat	Fruit	Fly
Cave wētā	2	1	5
Ground wētā	5	3	2
Cockroach	0	7	0
Total	7	11	7

Feeding

Three insect taxa were observed feeding in the food-choice arenas: Rhaphidophoridae cave wētā (*Isoplectron*); Blattidae cockroaches (*Celatoblatta*); Anostostomatidae ground wētā (*Hemiandrus*). A total of 25 independent observations were made (Table 6.4).

Hemiandrus were seen feeding on all three of the available foods (Fig 6.2A). In two instances, *Hemiandrus* individuals were observed eating oatmeal when the fly was gone, so these observations were not recorded, because consumption of the fly was not observed. Overall the cave wētā and the ground wētā ate at random, but the cockroaches showed a significant preference for the fruit ($P = 0.0009$ chi-square test). Although an important ecological association between ground wētā and fruit has been suggested (Burns 2006; but see Morgan-Richards et al. 2008), our data suggest that cockroaches might have a closer interaction with fruit than ground wētā, highlighting the limited understanding of native insect interactions with plants.

Population size estimation

On the first night 37 *H. maia* sp. nov. were marked at Kurinui, Otago (10 adult/subadult males; 7 adult females; 18 juveniles; 2 nymphs). On the second night, 73 wētā were observed (13 adult/subadult males; 16 adult females; 40 juveniles; 3 nymphs), of which three adult males were recaptures. We calculated population size to be 702±298, with a population density of 1.8 wētā/m^2.

On the first night, 15 *H. electra* sp. nov. were marked at Awaroa, Tasman (12 adult/subadult males; 3 adult females). On the second night, 12 wētā were observed (all adult/subadult males), one of which was a recapture. We calculated population size to be 103±52, with a population density of 0.3 wētā/m^2.

Conclusions

The Otago ground wētā, *H. maia* sp. nov., uses burrows during the day that are sealed with a new soil plug each time the wētā returns from nocturnal excursions. Even apparently inactive burrows contained wētā, and if we use the data from excavated burrows to extrapolate to the full set of burrows then all 75 probably contained live wētā, and about 20% might have had adult females with nymphs. We saw small holes (too small for adults to use) in nine (12%) of the burrow doors (Fig 6.1C). All burrows contained *H. maia* sp. nov. *Hemiandrus* "timaru" apparently have homes in other habitats, possibly the forest floor nearby.

During late December *H. maia* sp. nov. is, on average, active about once in every three nights. *Hemiandrus maia* sp. nov. feed on the ground where they eat invertebrates, fruit and seed contents. The females of *H. maia* sp. nov. share burrows with their eggs and nymphs. Nymphs exit and enter via small openings in the door of the burrow, even when the adult female does not emerge. Maternal care in this species has probably been independently derived, as this medium-ovipositor species is not closely related to short-ovipositor species in which this behaviour has previously been observed (Gwynne 2005). Many wētā die in their burrows, which are re-used. It is rare for a burrow entrance not to be re-sealed if a wētā is using it as a day-time hide.

Independent reduction of ovipositor length in the northern South Island wētā, *H. electra* sp. nov. might also be correlated with maternal care of eggs and nymphs. This prediction could be tested with a similar study of *H. electra* sp. nov. Sympatry with long-ovipositor species might drive selection for care of young; investigations to seek evidence of character displacement in this genus would be very rewarding.

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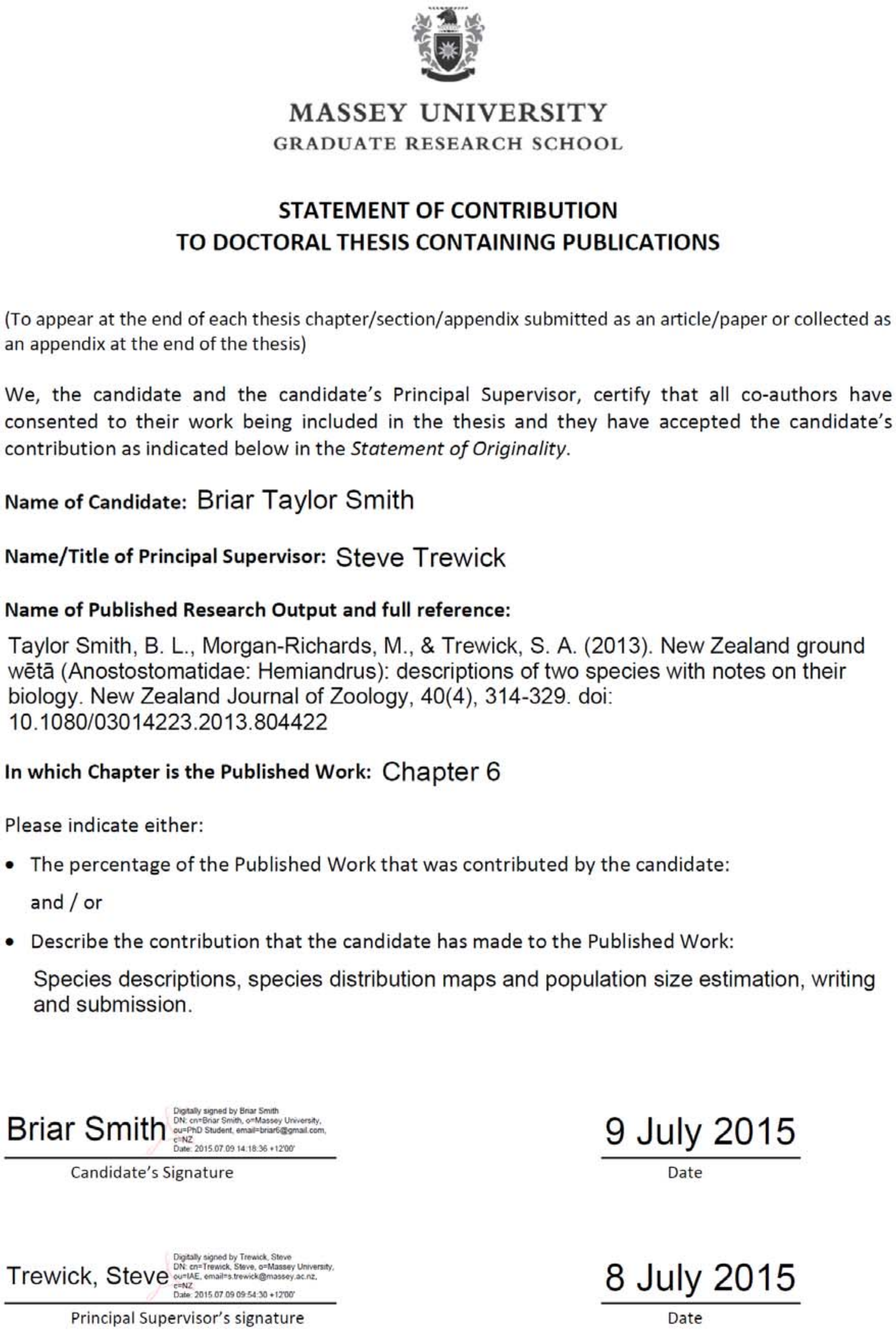

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Taylor Smith, B. L., Morgan-Richards, M., & Trewick, S. A. (2013). New Zealand ground wētā (Anostostomatidae: Hemiandrus): descriptions of two species with notes on their biology. New Zealand Journal of Zoology, 40(4), 314-329. doi: 10.1080/03014223.2013.804422

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Chapter 7. Evidence for two distinct lineages within *Hemiandrus*

Introduction

The currently described and tag-named[5] species of New Zealand ground wētā (*Hemiandrus*) encompass considerable morphological variation. A notable expression of this is in variation of size and shape of the ovipositor (egg-laying tube) of adult females. In some species the ovipositor is longer than the body, whilst in others it barely projects past the subgenital plate. This degree of difference prompted Salmon (1950) to split the genus into two with *Hemiandrus* for the short ovipositor species and *Zealandosandrus* for those species with long ovipositors. However, the observation that some species have intermediate length ovipositors caused Johns (1997) to synonymise these genera. Johns (2001) also found that modifications of the sixth abdominal sternite were present on females of the short ovipositor species and some (but not all) long ovipositor species too. The unification of these two genera was supported by mtDNA evidence showing that some closely related ground wētā taxa have extreme differences in ovipositor length that indicated multiple independent origins of shorter ovipositors i.e. convergent evolution (Pratt *et al.* 2008). However, exhaustive sampling of the New Zealand *Hemiandrus* and new phylogenetic analysis with mtDNA data resolves two well-supported clades within this genus (Fig 7.1: and see previous chapter). Of 22 *Hemiandrus* taxa included in the most recent analysis, eight group in Clade 1 and fourteen[6] in clade 2. Although ovipositor length alone does not discriminate these two clades there are indications that they may represent distinct evolutionary lineages.

Ground wētā are to be found throughout New Zealand in environments ranging from rainforest to coastal scrub. This diversity of habitats used by different species of *Hemiandrus* suggests adaptation to particular conditions. In addition, the presence of two or more species with different morphology in sympatry at numerous locations suggests there are fundamental ecological differences between these lineages that enable partitioning of resources. While studying the behaviour and ecology of nocturnal insects by direct observation is extremely challenging, morphological traits are informative about natural history. Here I explore whether the two mitochondrial clades identified in *Hemiandrus* might indeed represent distinct lineages, by testing whether they correspond with the partitioning of nuclear sequence data and

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[5] A tag-name is an informal name that indicates an entity that may be a separate species, monophyletic group or separate interbreeding population of uncertain taxonomic rank (Leschen *et al.* 2009).

[6] When *H. focalis* and *H. superba* are treated as same species.

morphological variation or whether all morphology varies independently of phylogenetic relationships.

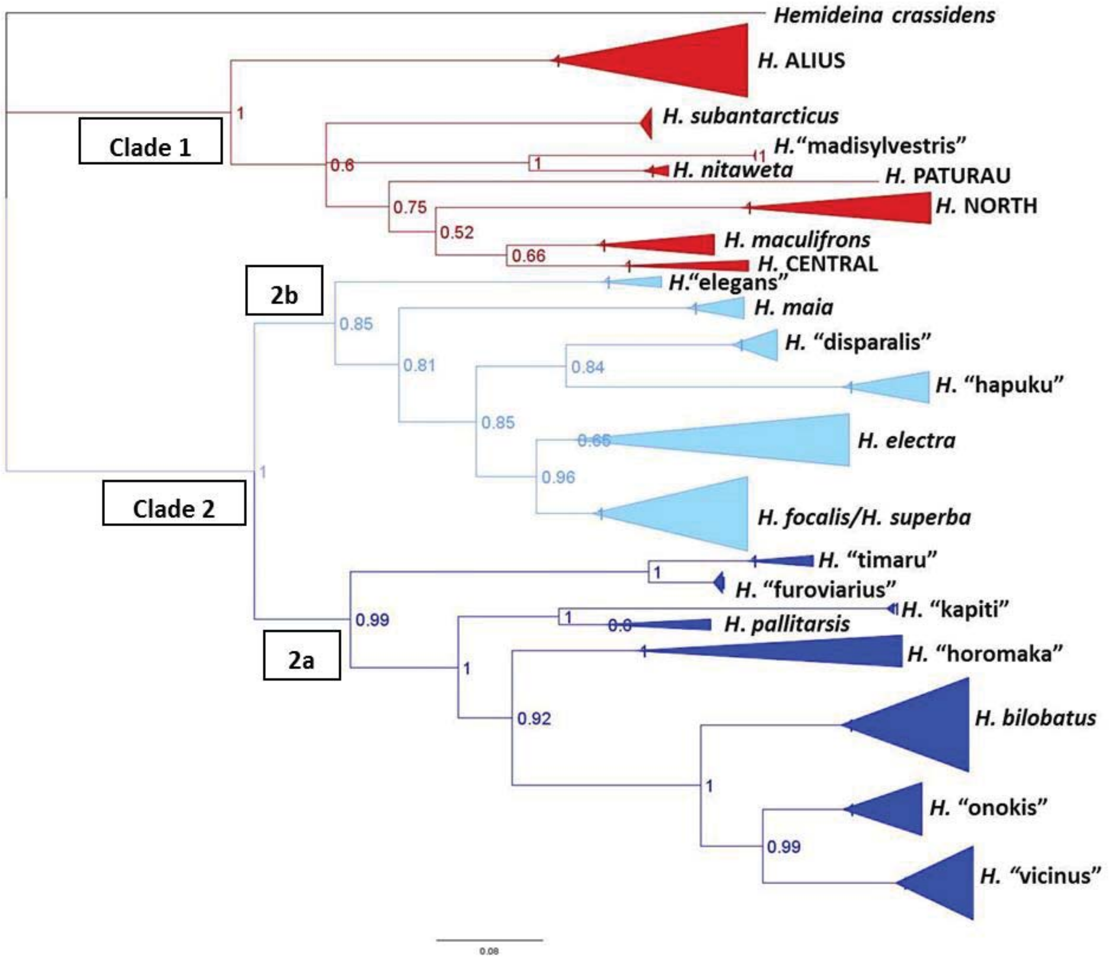


Figure 7.1. Bayesian mtDNA phylogeny of New Zealand *Hemiandrus* (based on 797bp of COI, from chapter 5). Numbers represent Bayesian posterior probabilities. MtDNA separates *Hemiandrus* into two clades: clade 1 (red) and clade 2 (blue). Clade 2 has a nested subclade (clade 2a, dark blue). The other clade 2 species (clades 2b, light blue) are either sister to or paraphyletic with respect to clade 2a. Species described in chapter 4 are treated here as described species but species names are given in capital letters to distinguish them from formally described species.

Methods

Molecular methods

Muscle tissue from a hind femur of each specimen was subjected to total genomic DNA extraction using a salting-out extraction method (Sunnucks and Hales 1996). Nuclear markers developed and described in chapter 2 were used to explore species evolutionary relationships. Three nuclear loci were amplified by polymerase chain reaction (PCR) using the following conditions: 94°C for 2 min then 35 cycles of 94°C for 30s, annealing at 56°C-59°C for 30s, and extension at 72°C for 30s; followed by 72°C for 8 min. Amplified DNA products were treated to Shrimp Alkaline Phosphotase⁄Exonuclease digestion (USB Corp.) to remove unincorporated primers prior to sequencing. Cycle sequencing with the PCR primers used Bigdye chemistry v3.1 (PE) following the manufacturer's protocols, with automated reading on an ABI3730. Sequences were edited and aligned using Geneious v6.1.6 (Kearse *et al.* 2012).

Phylogenetic analysis

Phylogenetic analyses of three nuclear loci (Appendix 7.1) and mtDNA (see chapter 5) used the most appropriate model of DNA evolution as evaluated using jModelTest v2.1.3 (Darriba *et al.* 2012). Bayesian phylogenetic analysis was performed in BEAST v1.8 (Drummond *et al.* 2012). Two BEAST analyses were run for 50 million generations each using a Yule tree prior, with a burn-in of 5 million generations for each run. Ambiguity codes were used to represent polymorphisms in heterozygous individuals and by editing the XML file, phylogenetic information in ambiguities was included in the BEAST analysis. Output statistics were examined using TRACER v1.5.0 (Rambaut and Drummond 2009). The runs were combined using LogCombiner v1.8 (Drummond *et al.* 2012) and trees were visualised using FigTree v1.4 (Rambaut 2012). The data was not concatenated to form a single tree because the same individuals were not used for all loci. A species tree was not created in *BEAST because sequences of all three loci were not available for all species.

Morphology

Qualitative traits

The shape of the female 6th–7th abdominal sternite was recorded. Male terminalia, which can evolve rapidly can provide useful characters for insect taxonomy (Eberhard 1985), so male terminalia shape was examined in 194 individuals encompassing 22 *Hemiandrus* taxa. The pilosity of the maxillary palps on the head, and patterning of the pronotum of all male and female specimens was examined and recorded. As no differences between sexes were detected, data were combined. SEM micrographs were used to compare the surface characteristics of eggs of different species. Eggs were attached to a mounting stub and sputter-coated with gold before being imaged with a FEI Quanta 200 scanning electron microscope (SEM, FEI Electron Optics, Eindhoven, The Netherlands) operated in back-scattering mode.

Quantitative traits

I took 10 measurements from 288 female *Hemiandrus* representing 24 tag-named or described species (Appendix 7.2). Specimens were examined using an Olympus SZX7 Zoom Stereomicroscope with an attached SC100 digital camera, and measured using digital callipers or Olympus Image Analysis Software. Measurements taken in millimetres were body length (BL: distance from frons to distal margin of metanotum), total body length (TBL: distance from frons to distal margin of ninth abdominal tergite), head width (HW), head length (HL), pronotum width (PW), pronotum length (PL), femur width (FW), femur length (FL), female ovipositor length (OV: length from anterior margin of the first valvula to the tip of the ovipositor) (Fig. 7.2) and egg length (EL). Pronotum shape (length divided by width) was calculated. Ovipositor length was scaled to head width to give relative ovipositor length.

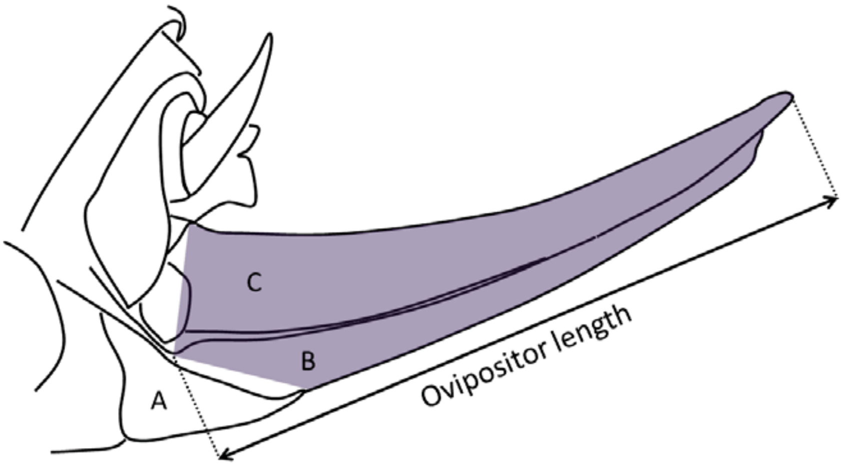


Figure 7.2. Female ovipositor length of *Hemiandrus* was measured from base to tip. The shaded area represents the part subject to shape analyses by elliptic Fourier analysis. A) Subgenital plate; B) first valvula; C) third valvula.

Using female specimens, I counted hind tibial spines including both the inferior articulated spines and the superior fixed spines on prolateral and retrolateral angles (Fig. 7.3). Spines on both legs of the pair were counted and averaged for each individual. I counted stridulatory pegs on the inner hind femur and first 3 abdominal tergites on the dextral side only. When counting

T1-T3 pegs I recorded exact numbers unless there were > 100 pegs due to the error associated with counting large numbers.

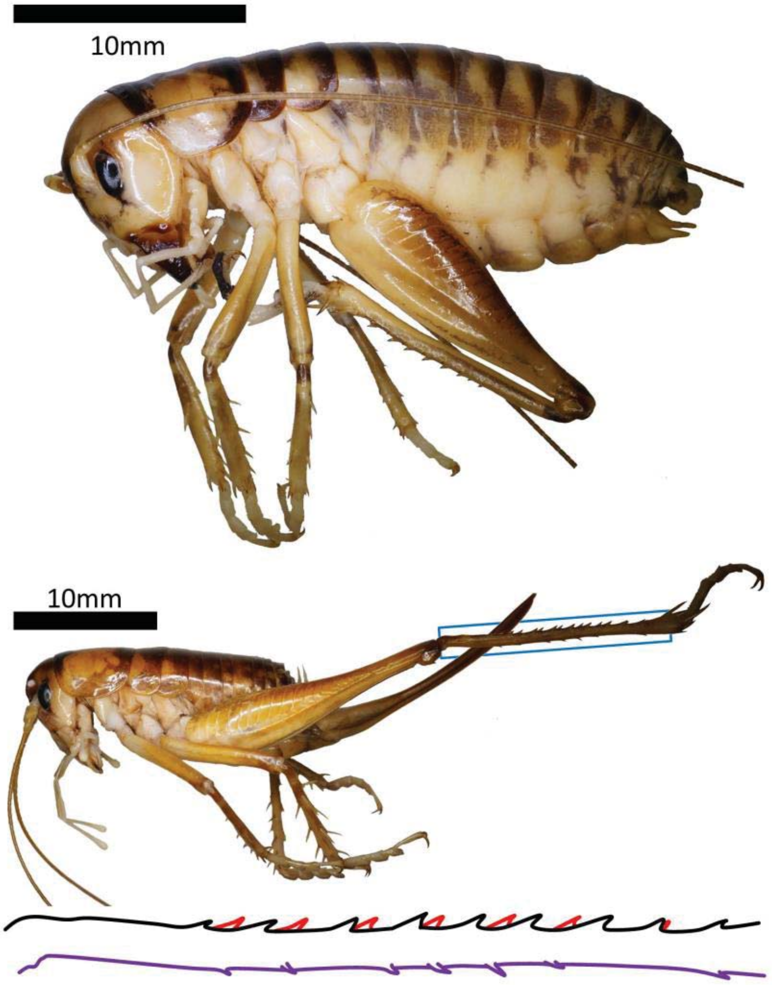


Figure 7.3. Ovipositor length variation in *Hemiandrus* species relative to body size. Ovipositors range from barely projecting past the subgenital plate e.g. *H.* “vicinus” (top) to longer than body length e.g. *H.* “disparalis” (bottom). The hind tibia of *H.* “disparalis” (blue box) is enlarged to show the positions of the hind tibial spines. The fixed spines of the superior hind tibia include the prolateral spines (black) and the retrolateral spines (red). The inferior side of the hind femur has articulated spines (purple).

Average values were calculated for each variable in each species. Species averages were inspected using principal component analysis (PCA) using the R package 2.15.1 (R Development Core Team 2010). Species were assigned to one of two clades based on mtDNA evidence. Clade associated traits were compared using t-tests on species averages. Not all measurements were available for all individuals due to damaged specimens or an absence of/inability to extract eggs so sample sizes vary.

Geometric morphometric analysis of ovipositor shape

To control for the influence of size differences on shape, I assessed variation in ovipositor shape using geometric morphometric (GMM) analysis. I photographed the lateral view of the ovipositors of 67 females excluding species with very short ovipositors or damaged ovipositors. The shape recorded for outline analysis terminates ventrally at the anterior of the ovipositor where the subgenital plate meets and first valvula, and dorsally on the 3rd valvula perpendicular to the margin of the first valvula (Fig. 7.2). Shapes were digitised using tpsDIG v2.16 (Rohlf 2010). Using the software package SHAPE v1.3 (Iwata and Ukai 2002), I performed an elliptic Fourier analysis (EFA) (McLellan and Endler 1998) which reduces two-dimensional outlines into elliptic Fourier descriptors (EFDs) that can then be analysed statistically (Kuhl and Giardina 1982). The contour shape was described in the first 20 harmonics of Fourier coefficients. Principal component analysis was performed on the resulting normalised EFD coefficients.

Results

Genetic support for two clades

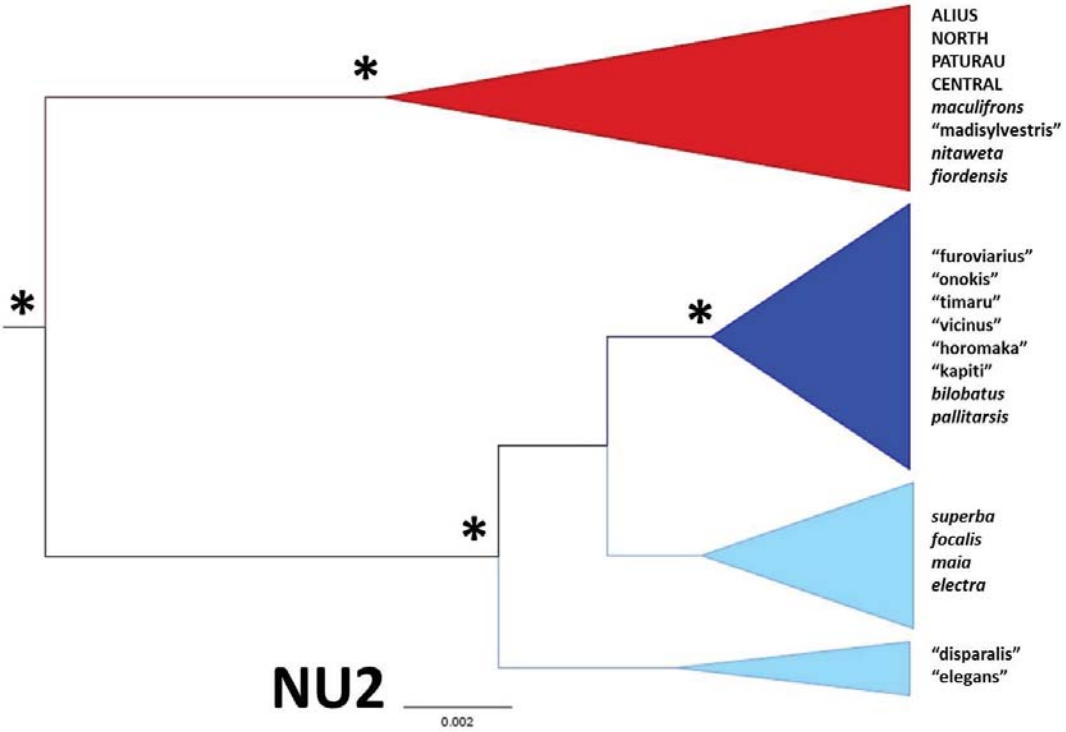
Mitochondrial DNA sequences resolved phylogenetic relationships of *Hemiandrus* with eight species in one clade and 14 species in another clade. These are referred to as clade 1 and clade 2 respectively (Table 7.1). See previous chapter for additional details.

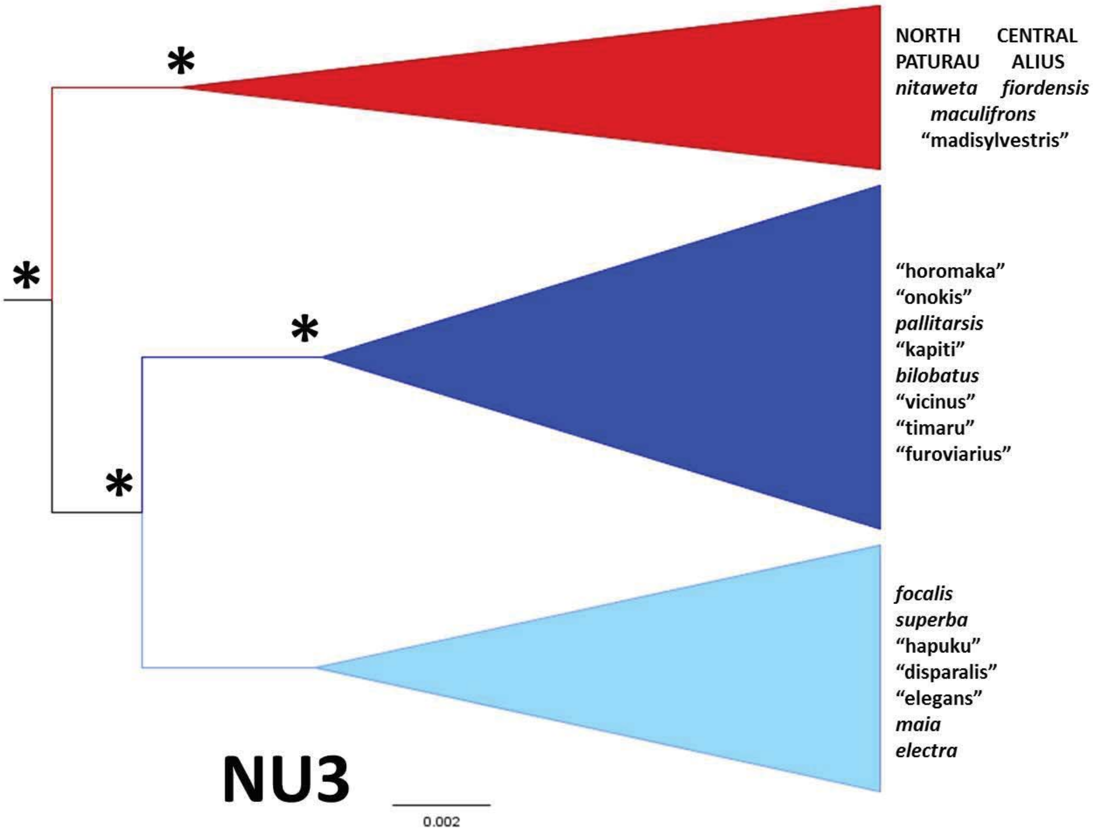
Table 7.1. Numbers of *Hemiandrus* females measured and males examined for terminalia shape in each species. Clade membership of each species and mean ovipositor length as a percentage of total body length are indicated. *includes *H.* "promontorius"; **included in "short" clade but has a long ovipositor; ***includes both North Island and South Island.

Name	Females	Males	mtDNA	Nuclear loci (NU2 NU3 NU13)	Clade	Proportional mean ovipositor length (of total body length)
subantarcticus	2	3	Y	NU13	1	54%
fiordensis	4	2	N	NU2 NU3	1	59%
ALIUS***	26	15	Y	NU2 NU3 NU13	1	60%
nitaweta	4	2	Y	NU2 NU3	1	61%
"madisylvestris"	3	0	Y	NU2 NU3	1	66%
CENTRAL	34	9	Y	NU2 NU3 NU13	1	67%
NORTH	81	33	Y	NU2 NU3 NU13	1	71%
PATURAU	1	1	Y	NU2 NU3 NU13	1	79%
maculifrons	63	15	Y	NU2 NU3 NU13	1	79%
"saxatilis"	0	1	N	No	1	NA
electra	11	17	Y	NU2 NU3 NU13	2b	30%
maia	13	12	Y	NU2 NU3 NU13	2b	34%
"hapuku"	4	7	Y	NU3	2b	42%
superba	1	5	Y	NU2 NU3 NU13	2b	59%
focalis	3	4	Y	NU2 NU3 NU13	2b	65%
"elegans"	1	2	Y	NU2 NU3	2b	78%
"disparalis"	7	4	Y	NU2 NU3 NU13	2b	94%
"horomaka"	6	11	Y	NU2 NU3 NU13	2a	7%
pallitarsis	5	10	Y	NU2 NU3 NU13	2a	10%
"vicinus"	4	20	Y	NU2 NU3 NU13	2a	11%
*bilobatus**	8	9	Y	NU2 NU3 NU13	2a	11%
"onokis"	3	4	Y	NU2 NU3 NU13	2a	12%
"furoviarius"	0	0	Y	NU2 NU3	2a	NA
"kapiti"	1	8	Y	NU2 NU3 NU13	2a	Not recorded
"timaru"	3	0	Y	NU2 NU3 NU13	2a **	62%
Total	288	194	23	24	NA	NA

I analysed three nuclear loci (see chapter 2). Despite relatively short sequence length (194 -217 bp) of these loci, the level of polymorphism was sufficient to resolve many species relationships. The NU2 dataset comprised 53 sequences from 22 *Hemiandrus* species. The alignment of 209 bp contained 30 (14.4%) variable, and 28 (13.4%) parsimony informative positions. The NU3 dataset comprised 49 sequences from 23 *Hemiandrus* species. The 217 bp alignment consisted of 36 (16.6%) variable, and 28 (12.9%) parsimony informative positions. The NU13 dataset comprised 41 sequences from 18 *Hemiandrus* species and two *Hemideina* sequences as an outgroup. This alignment of 43 sequences was 194 bp contained 50 (25.8%) variable, and 41 (21.1%) parsimony informative positions. For each of these three loci some alleles were shared among species, but many species had unique alleles.

The nuclear Bayesian phylogenies (Fig. 7.4) supported *Hemiandrus* as comprising two distinct, well-supported clades, comprising the same species as determined by mtDNA analyses. NU2 and NU3 were amplified for the additional species, *H. fiordensis*, and showed that it falls within clade 1 (for these loci; NU2, NU3). Furthermore, nuclear and mitochondrial data both supported a subclade within clade 2 (clade 2a) comprising eight species: *H. bilobatus*, *H. pallitarsis*, *H.* “timaru", *H.* “furoviarius”, *H.* “kapiti”, *H.* “horomaka”, *H.* “onokis” and *H.* “vicinus”. The evolutionary relationships among the other seven clade 2 species were not fully resolved. In the phylogenies inferred from nuclear markers they tend to be paraphyletic with respect to clade 2a. In the mtDNA analysis, sequences formed a clade but with low support but for convenience these species will be referred to here as clade 2b. Deeper nodes in the phylogeny were well-supported but shallower nodes were not and so are not shown.


*
*
*
*
ALIUS
NORTH
PATURAU
CENTRAL
maculifrons
"madisylvestris"
nitaweta
fiordensis
"furoviarius"
"onokis"
"timaru"
"vicinus"
"horomaka"
"kapiti"
bilobatus
pallitarsis
superba
focalis
maia
electra
"disparalis"
"elegans"
NU2
0.002


*
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NORTH CENTRAL
PATURAU ALIUS
nitaweta fiordensis
maculifrons
"madisylvestris"
"horomaka"
"onokis"
pallitarsis
"kapiti"
bilobatus
"vicinus"
"timaru"
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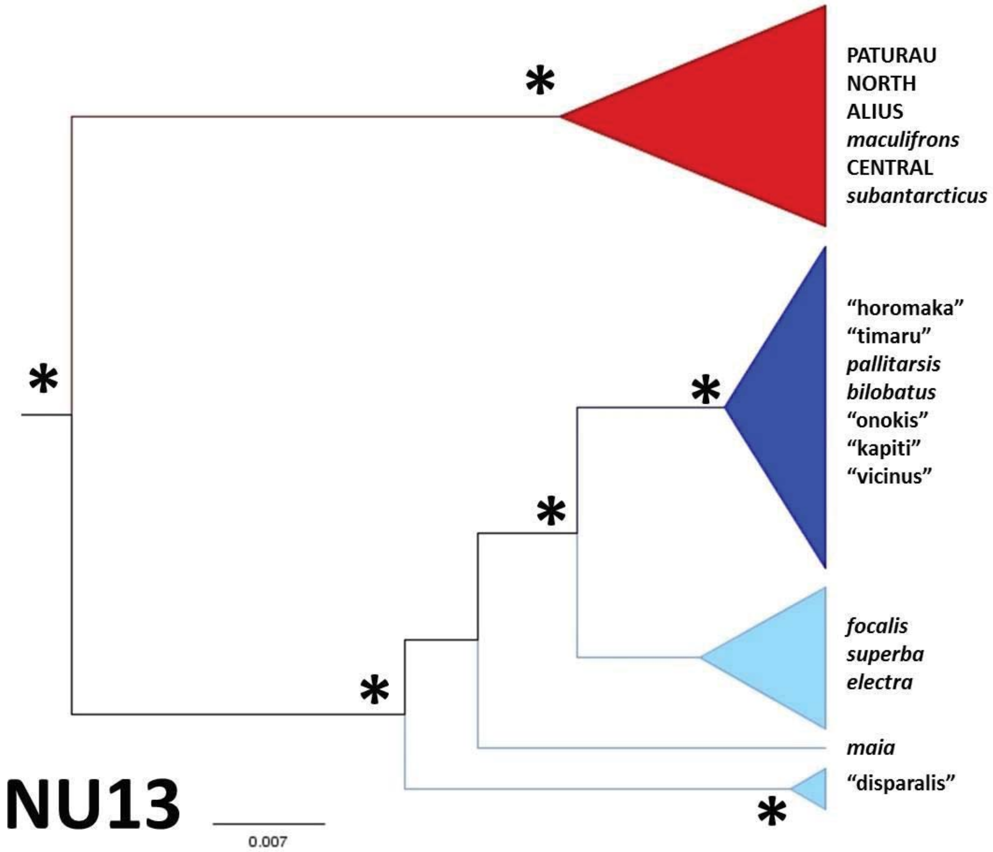

Figure 7.4. Bayesian phylogenies of three nuclear loci from representatives of *Hemiandrus*. Stars indicate all nodes with posterior probabilities of ≥0.95. All three loci support the monophyly of clade 1 species (red), clade 2 species (blue) and within this latter clade, clade 2a (dark blue). Most clade 2a species have reduced ovipositors but *H.* "timaru" has a long ovipositor. Note that NU13 included *Hemideina* spp as outgroup (not shown).

Morphology

Qualitative traits

Traits of both sexes

All species in clade 1 had small hairs covering the entire 4th segment and usually part of the 3rd segment of the maxillary palps (Fig. 7.5). In clade 2 species, the 4th segment was never entirely clothed in these small hairs, and hairs were absent from the 3rd segment (Fig. 7.6).

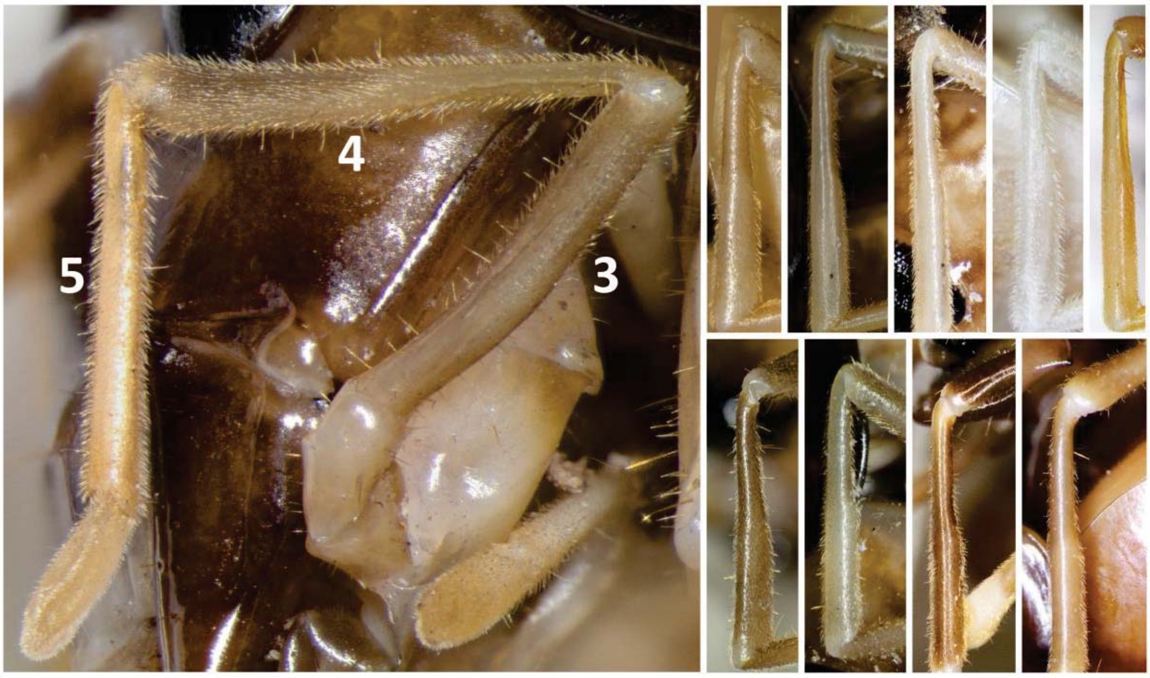

Figure 7.5. All species in the clade 1 have small hairs covering the whole of the 4th segment of the maxillary palps and usually part of the 3rd segment. Enlarged picture: *H.* ALIUS; third, fourth and fifth segments are numbered. Smaller pictures, top row: *H. maculifrons*, *H.* “madisylvestris”, *H.* PATURAU, *H.* “saxatilis”, *H. subantarcticus*. Bottom row: *H.* NORTH, *H.* CENTRAL, *H. nitaweta*, *H. fiordensis*.

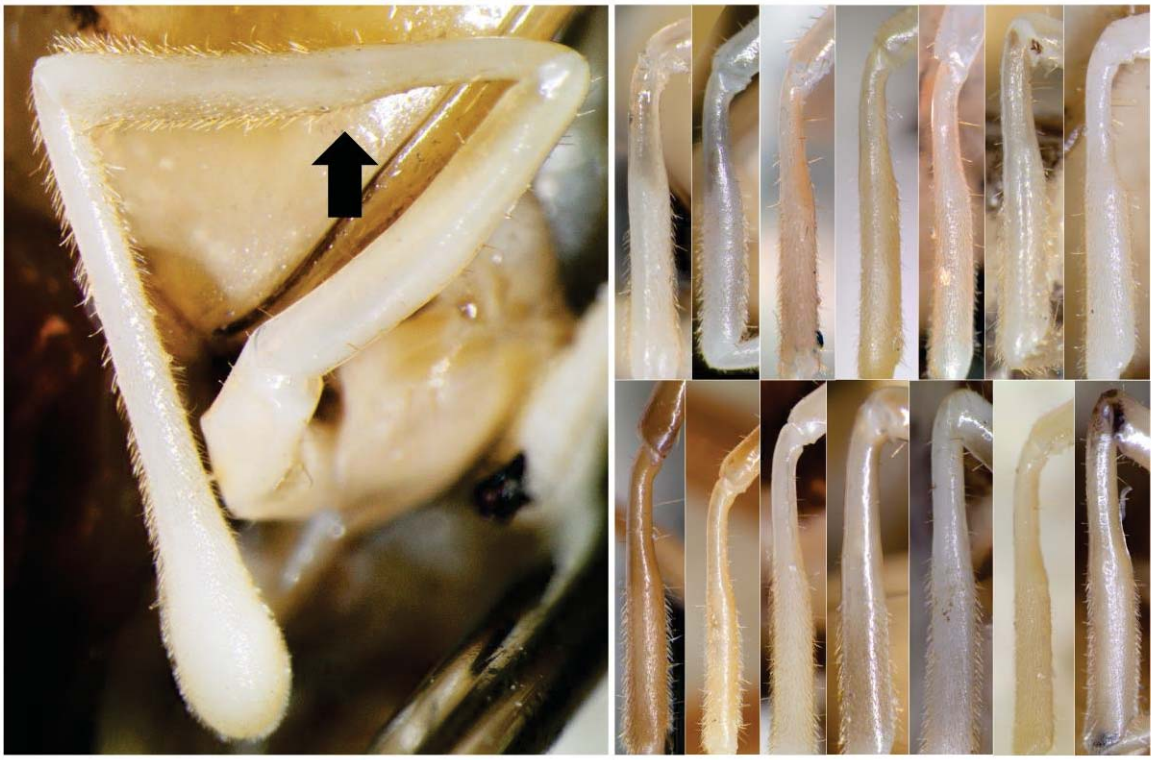

Figure 7.6. In clade 2, the 4th segment of the maxillary palps is never completely covered in small hairs and hair is lacking from the 3rd segment. Enlarged picture: *H. pallitarsis*; the black arrow indicates the approximate endpoint of the pilose region of the fourth segment. Smaller pictures, top row: *H.* “furoviarius”, *H.* “horomaka”, *H.* “kapiti”, *H.* “onokis”, *H. bilobatus*, *H.* “timaru”, *H. vicinus*. Bottom row: *H.* “disparalis”, *H.* “elegans”, *H. electra*, *H. focalis*, *H.* “hapuku”, *H. maia*, *H. superba*.

The patterning of the pronota also varied between species of the two clades. With the exception of *H. focalis* and *H. superba*, clade 2 species had large lateral pale patches extending nearly to midline (Fig. 7.7). *Hemiandrus focalis* and *H. superba* had very wide pronota which differed in texture and pattern to all other species. Large lateral pale patches were absent from clade 1 species except for *H. nitaweta*. Clade 1 species had smaller patches or no pronotum patterning.

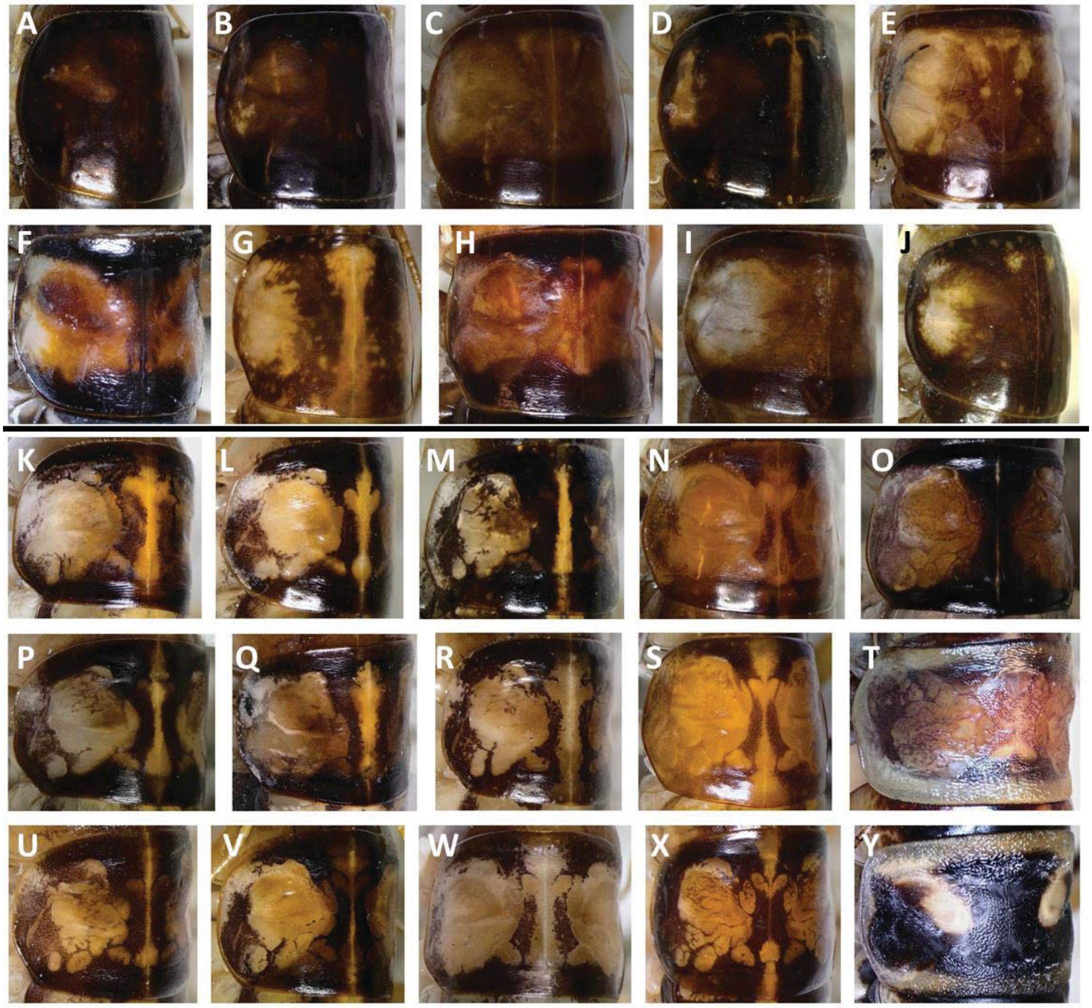

Figure 7.7. The two *Hemiandrus* clades differ in both pronotum shape and pronotum pattern. Clade 1 pronota (top two rows) tend to be quite square, while those of clade 2 (bottom three rows) are broader. A) *H.* ALIUS. B) *H.* "madisylvestris". C) *H.* PATURAU. D) *H.* NORTH. E) *H. subantarcticus*. F) *H. nitaweta*. G) *H. maculifrons*. H) *H. fiordensis*. I) *H.* "saxatilis". J) *H.* CENTRAL. K) *H.* "vicinus". L) *H.* "kapiti". M) *H.* "timaru". N) *H.* "elegans". O) *H.* "hapuku". P) *H. bilobatus*. Q) *H.* "onokis". R) *H.* "horomaka" (Kaikoura). S) *H.* "disparalis". T) *H. superba*. U) *H. pallitarsis*. V) *H. maia*. W) *H.* "horomaka". X) *H. electra*. Y) *H. focalis*.

Males

The species of the two clades differed consistently in the shape of male terminalia (Fig. 7.8, 7.9). Clade 1 males had distinct but adjacent falci that were usually touching (Fig. 7.9B). Their falci tended to be elongated and knife-shaped, while their paraprocts were soft lobes. Clade 2 males had knob-like falci positioned apart and pointed paraprocts (Fig. 7.9A).

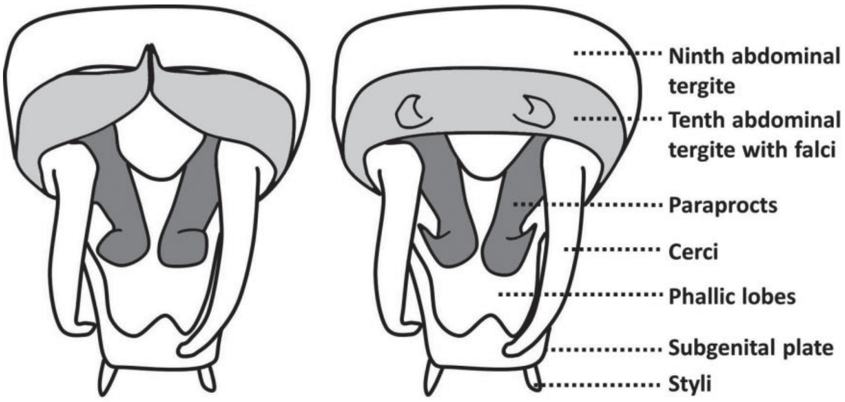

Figure 7.8. Two distinct types of adult male terminalia in *Hemiandrus*. Clade 1 species (left) have distinct adjacent falci (sharp and knife-shaped) and soft paranal process. Clade 2 species (right) have terminalia with widely spaced knob-like falci and sharp paranal processes. During mating the paraprocts and falci fit around or into the sternite modifications of females and may function to force copulation or ensure the correct alignment of genitalia. Once attached, the phallic lobes deliver the spermatophylax and sperm ampulla.

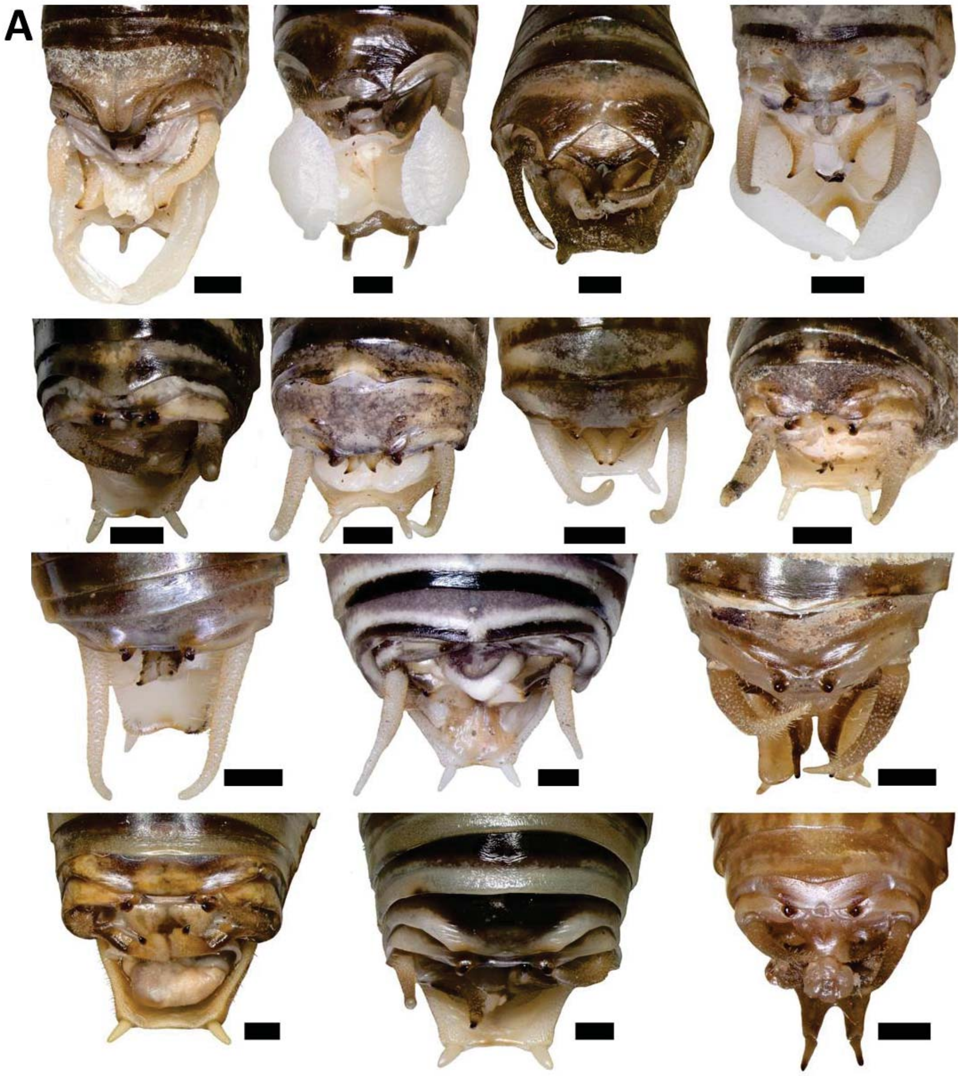

Figure 7.9. A. Male terminalia of clade 2 *Hemiandrus* species. *Hemiandrus pallitarsis*, *H. bilobatus* (North Island), *H. bilobatus* (South Island), “onokis”, *maia*, “vicinus”, “horomaka”, “Kapiti”, *electra*, “Hapuku”, “elegans”, *focalis*, *superba*, “disparalis”. **B**. Male terminalia of clade 1 species. *Hemiandrus* NORTH, CENTRAL, *maculifrons*, PATURAU, “saxatilis”, ALIUS, *subantarcticus*, *fiordensis*, *nitaweta*. Scale bar represents 1mm. Note that *pallitarsis*, *bilobatus*, and “onokis” have inflated phallic lobes in these pictures. Note that I do not have DNA for “saxatilis” but its terminalia and maxillary palp pilosity indicates that it is part of long clade.

Females

Hemiandrus species in the two main mtDNA clades differed in terms of female sternite modifications (Fig. 7.10). All species in clade 2 had modified 6th sternites, whereas those in clade 1 had unmodified 6th sternites but a pair of pits situated near the anterior edge of the 7th sternite either side of the midline. Sixth sternite modifications of clade 2 species included either lobes or pits along the posterior margin of the sternite. The lobes range in shape and size both within but particularly between species. Most species in clade 2a clade had large lobes, whereas *H*. “kapiti” had small pits similar to those of species in clade 2b (Fig. 7.10H).

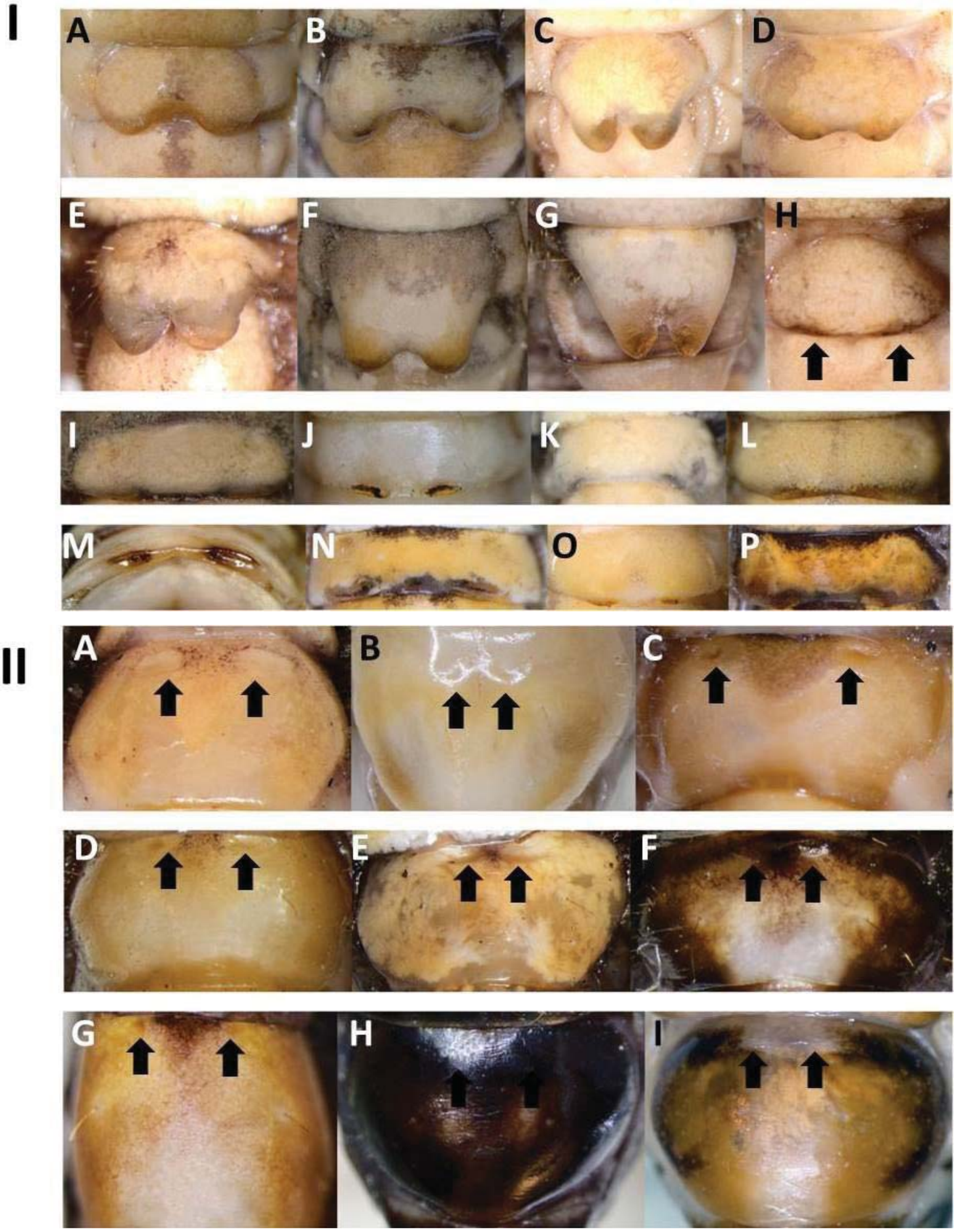

Figure 7.10. Ventral view of female sternite shape **I**. Clade 2 species. A) *H.* "horomaka" (Kaikoura). B) *H.* "horomaka" (Banks Peninsula). C) *H. bilobatus* (North Island). D) *H.* "vicinus". E) *H. pallitarsis*. F) *H.* "onokis". G) *H. bilobatus* (South Island). H) *H.* "kapiti". I) *H.* "hapuku". J) *H. electra*. K) *H.* "timaru". L) *H. maia*. M) *H. focalis* (anterior view showing pockets). N) *H. focalis* (ventral). O) *H.* "disparalis". P) *H. superba***.** **II.** Clade 1 species. A) *H.* PATURAU. B) *H. maculifrons.* C) *H. subantarcticus*. D) *H.* NORTH. E) *H.* CENTRAL. F) *H.* ALIUS. G) *H.* "madisylvestris". H) *H. nitaweta*. I) *H. fiordensis*.

Eggs were examined for chorion structure. The eggs were extracted unlaid from preserved females resulting in artefacts which obscured some of the chorionic structure (e.g. Fig. 7.11 *H.*

ALIUS). New images gathered here for *H. maculifrons* and *H. subantarcticus* present chorion structures that match the SEM micrographs of mature, laid eggs photographed by Cary (1981) and Butts (1983) respectively, indicating that the patterns on unlaid eggs are representative of patterns on laid eggs. All species in clade 2a that had short ovipositors had simple chorion structure (Fig. 7.11, Table 7.2) and although not visible at the magnification used it is likely that respiratory pores (aeropyles) were present. Most other species in clade 2 had a chorion with an irregular array of pores with a maximum diameter of 10-20μm, whereas eggs of *H.* "disparalis" had pores arranged along small ridges. Clade 1 *Hemiandrus* had a variety of chorion forms, with eggs of most species having had a ridged chorion either with or without large basal pores (20-30 μm), but *H.* ALIUS[7] and *H. subantarcticus* each had a chorion covered with large pores (30-40 μm), and no ridges, similar to the chorion of tree wētā, the outgroup species.

Quantitative traits

Species in clades 1 and 2 did not differ significantly in any of the body measurements (Appendix 7.2) (*t*-test, $p>0.05$), but they did differ in pronotum shape (*t*-test, $p < 0.001$); clade 2 species had wide pronota (mean ratio=0.85±0.05), whereas those of clade 1 species tended to be more square (mean ratio=0.97±0.05) (Fig. 7.12). There was a significant difference in the average relative ovipositor length (scaled to head width) of species in the two clades ($p=0.006$) (Fig. 7.13, 7.14). Clade 1 species had long ovipositors between 51 and 83% of total body length, whereas clade 2 consisted of species with ovipositors between 6% and 104% of total body length. There was a strong significant positive relationship between body size and ovipositor length in clade 1 ($R^2=0.88$), but a weaker relationship among the clade 2 species ($R^2=0.43$; Fig. 7.15). Six of the seven species in clade 2a examined for morphology had very short ovipositors, the exception being *H.* "timaru".

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[7] Species described in chapter 4 are treated here as described species but species names are given in capital letters to distinguish them from formally described species.

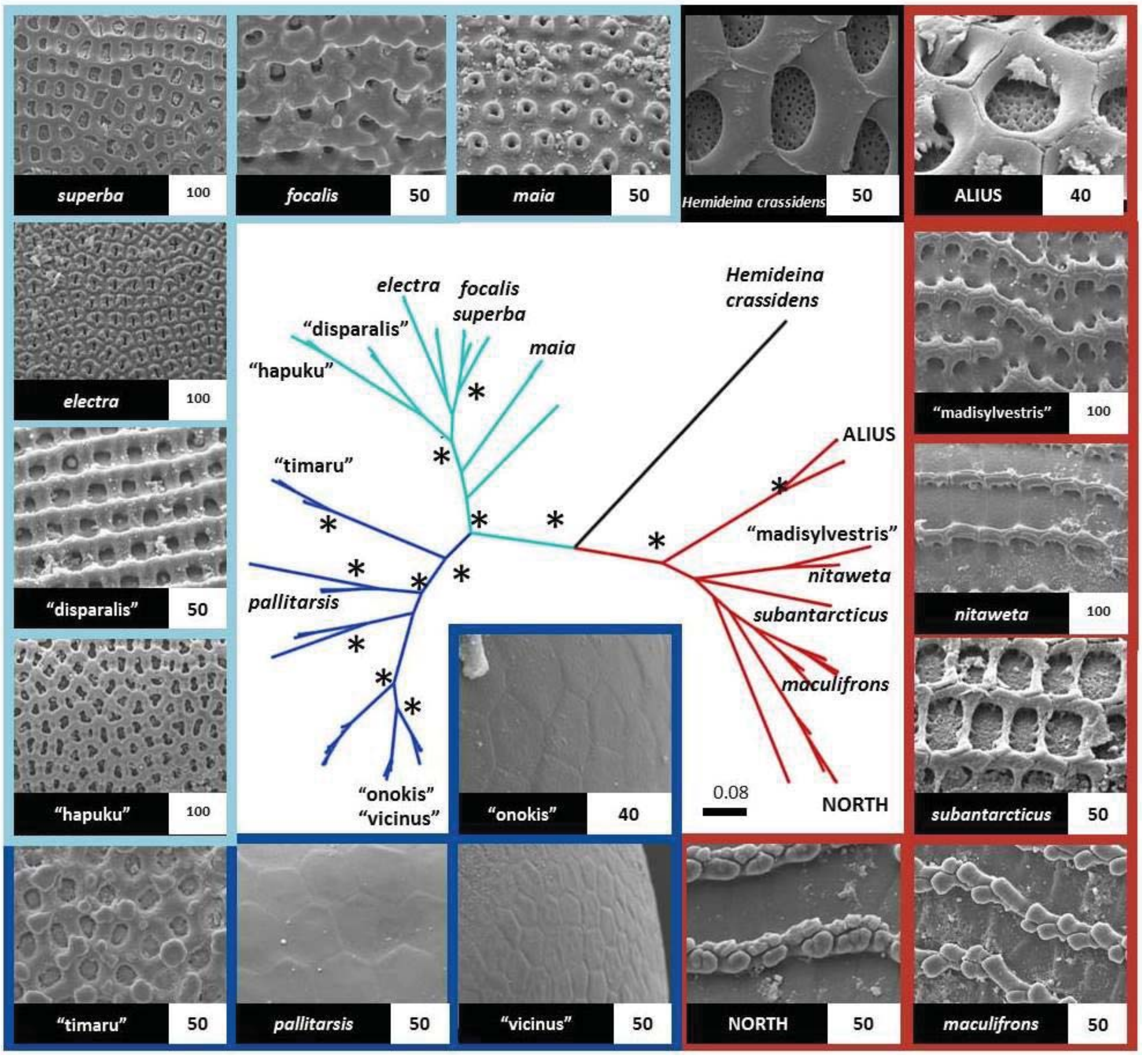
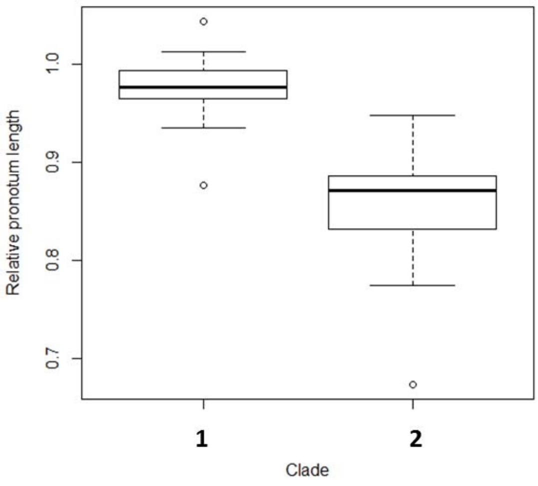
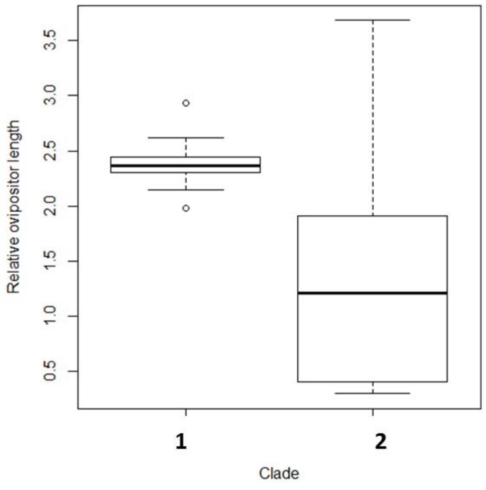


Figure 7.11. Chorion structure of eggs from 16 *Hemiandrus* species. Border colours indicate which of three mtDNA clades species are associated with (see Fig. 7.1). Species with short ovipositors (*H. pallitarsis*, *H.* "vicinus", *H.* "onokis", *H.* "Kapiti"-not shown) had simple chorion structure (dark blue), but *H.* "timaru" had chorion structure more like those of the rest of clade 2 (light blue) i.e. irregularly positioned pores with a maximum diameter of 10-20µm. The pores of *H.* "disparalis" were arranged to form small ridges. *Hemiandrus* ALIUS and *H. subantarcticus* had a chorion covered with large pores (30-40 µm), and no ridges, similar to the chorion of tree wētā (black), the outgroup species. Other species in clade 1 had a ridged chorion. Only species with eggs illustrated are labelled on the mtDNA phylogeny (from chapter 5). The stars indicate nodes with prior probabilities of >0.95. The white box associated with each picture is the scale bar with the value indicating the size in micrometres.

Table 7.2. Chorion morphology of *Hemiandrus* eggs.

Species	Main surface feature	Maximum diameter of large pores (μm)
H. pallitarsis	Smooth with shallow polygons	NA
H. “vicinus”	Smooth with shallow polygons	NA
H. “onokis”	Smooth with shallow polygons	NA
H. maia	Pores	10
H. electra	Pores	15
H. “hapuku”	Pores	20
H. “timaru”	Pores	20
H. focalis	Pores	20
H. superba	Pores	20
H. “disparalis”	Pores arranged to form ridges	15
H. “madisylvestris”	Ridges with pores at base	30
H. nitaweta	Ridges with pores at base	20
H. fiordensis	Ridges with pores at base	30
H. maculifrons	Ridges	NA
H. NORTH	Ridges	NA
H. ALIUS	Pores	30
H. subantarcticus	Pores	40
Hemideina sp.	Pores	60





Left: Figure 7.12. The two *Hemiandrus* clades differed in terms of pronotum shape (pronotum length divided by pronotum width) (t-test, $p<0.001$); clade 2 species had wide pronota (mean=0.85±0.05), whereas those of clade 1 tended to be more square (mean=0.97±0.05). Clade 1 species n=9 species, clade 2 species n=14 species.

Right: Figure 7.13. The two *Hemiandrus* clades different in terms of relative ovipositor length (scaled to head width) (t-test, $p=0.006$). Clade 1, n=9 species; clade 2, n=13 species.

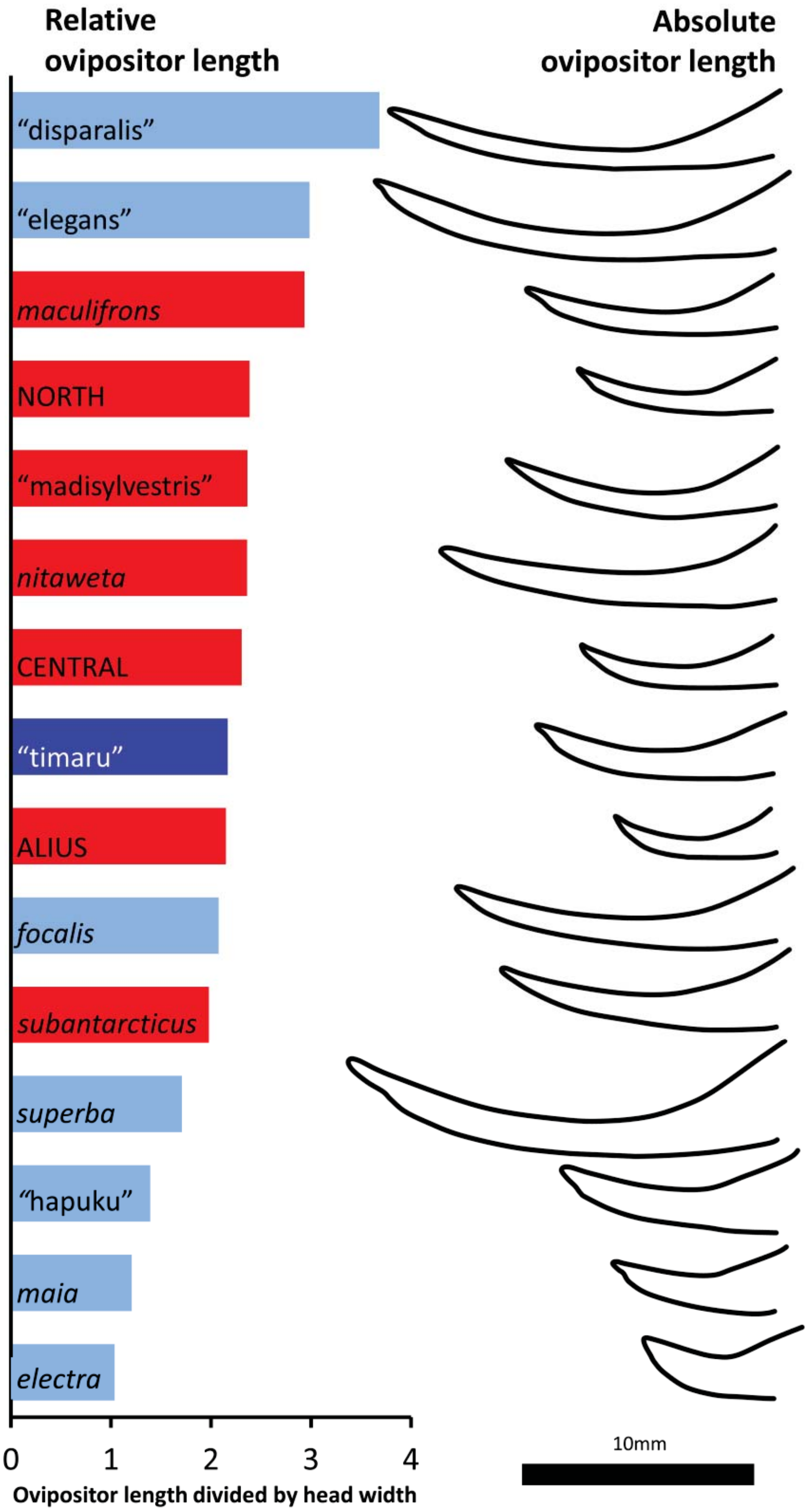


Figure 7.14. Ovipositor lengths of *Hemiandrus* species. Included here are only those with ovipositors more than 20% of body length. On the left is relative ovipositor length (ovipositor length divided by head width). On the right is the absolute length and shape of the ovipositor (rotated to longest radius of first harmonic). Clade 1 species names in red, clade 2a species in dark blue, clade 2b species in light blue. Clade 2 species included in this figure are only those with ovipositor lengths ≥20% of total body length.

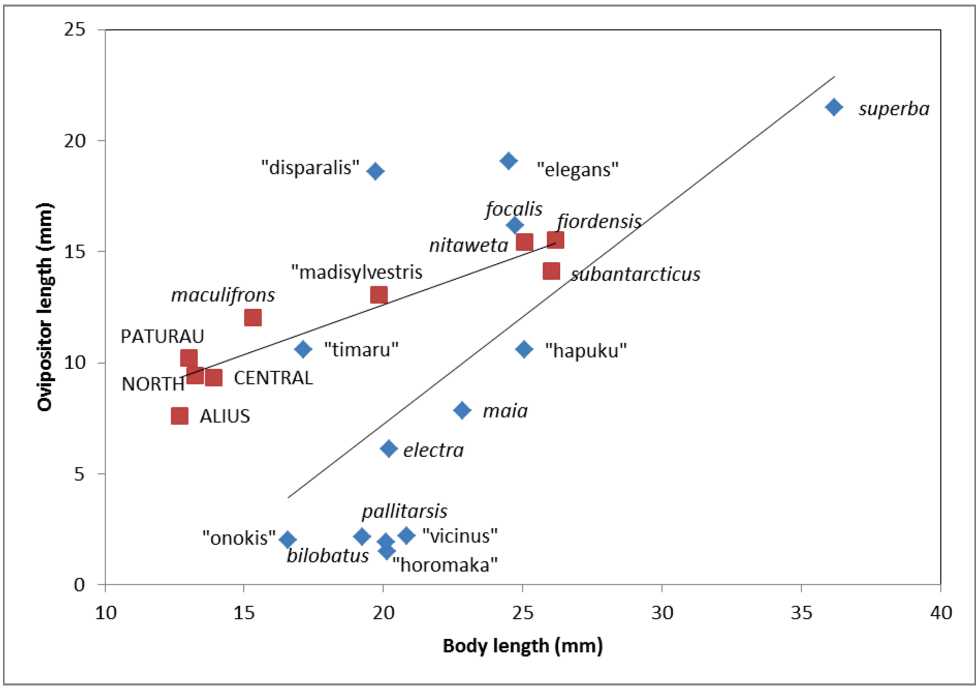


Figure 7.15. There is a strong significant positive relationship between *Hemiandrus* body size and ovipositor length in clade 1 (red) (R^2=0.88), but a weaker relationship clade 2 (blue) (R^2=0.43).

Species in clade 1 had more spines on the superior prolateral (mean=13±3; t-test, $p < 0.001$) and retrolateral angles (mean=13±3; t-test, p=0.001) of the hind tibiae than clade 2 species (prolateral = 8±1, retrolateral = 8±0), but fewer on the inferior side of the hind tibiae (mean = 2±1, variable clade mean 4±2; t-test, p = 0.01). *Hemiandrus nitaweta* was, however, an outlier with fewer spines on the superior hind tibiae. Members of clade 2 had more pegs on the inner side of the hind femur (mean=77±39) than clade 1 species (mean=22±16; t-test, $p< 0.001$). Clade 2 species had more pegs on T1-3 (Appendix 7.3).

Principal component analysis of measured and counted variables supported the separation of clades 1 and 2 species. The first two principal components, PC1 and PC2, explained 49.65% and 34.03% of the total variance, respectively (Fig. 7.16). All characters except for superior hind tibial spine number had negative loadings on PC1, for which the highest loadings were associated with body size measurements and the number of inferior hind tibial spines (Appendix 7.4). The highest loadings on PC2 were stridulatory peg number (both tergal and femoral) and superior hind tibial spine number, which were negative and positive respectively. Ovipositor length also had a high positive loading. The two clades overlapped in PC1 indicating that they are similar in terms of body size and inferior hind tibial spines but they did not overlap in PC2 scores indicating that pegs, superior spines and ovipositor length differed between the two clades.

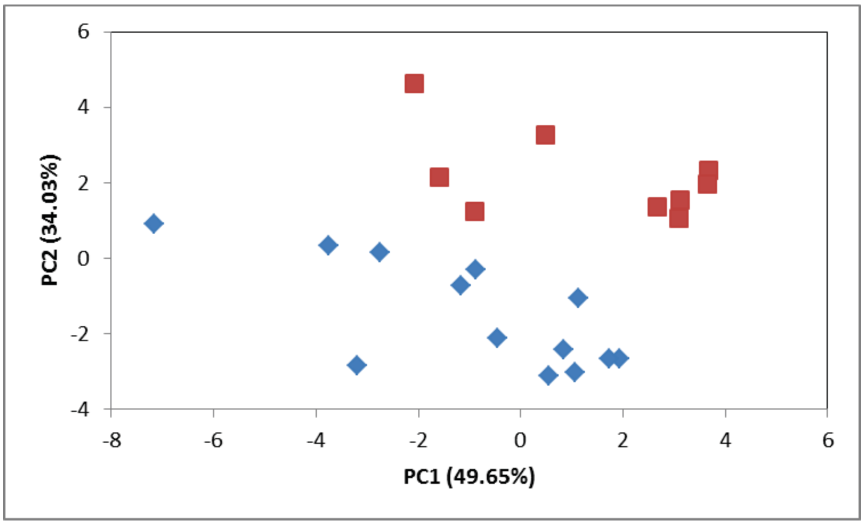


Figure 7.16. Plot of PCA components of all body measures (excluding egg size and total body length because not available for all species), spine counts and peg counts of *Hemiandrus* clearly separated the two clades. The first and second principal components explained 83.68% of the total variance. Clade 1 is red, clade 2 is blue. *H.* "kapiti" was excluded because of a lack of data.

Ovipositor shape variation was explored in species whose ovipositors are more than 20% of the total body length, so excluded clade 2a species except for *H.* "timaru". Principal component analysis of ovipositor shape showed that the first and second principal components explained 92.6% of the total variance (Fig. 7.17). The first PC was strongly correlated with ovipositor length (R^2=0.92) and described the shape change associated with ovipositor length i.e. long ovipositors were skinny, while short ovipositors were quite fat and stumpy (Fig. 7.18). The first PC divided clade 2 species into two with the species *H. maia*, *H. electra* and *H.* "Hapuku" falling together (Fig. 7.19). These species likely all have maternal care: *H. maia* (Taylor Smith *et al.* 2013) and *H.* "Hapuku" (pers obs) females have been observed to be sharing a burrow with nymphs, and *H. electra* has been observed with eggs in her burrow (SAT, MMR). The second PC represented ovipositor curvature, and in clade 1 it was correlated with ovipositor length (R^2=0.5, p<0.000) meaning that longer ovipositors were straighter, while shorter ones were more curved.

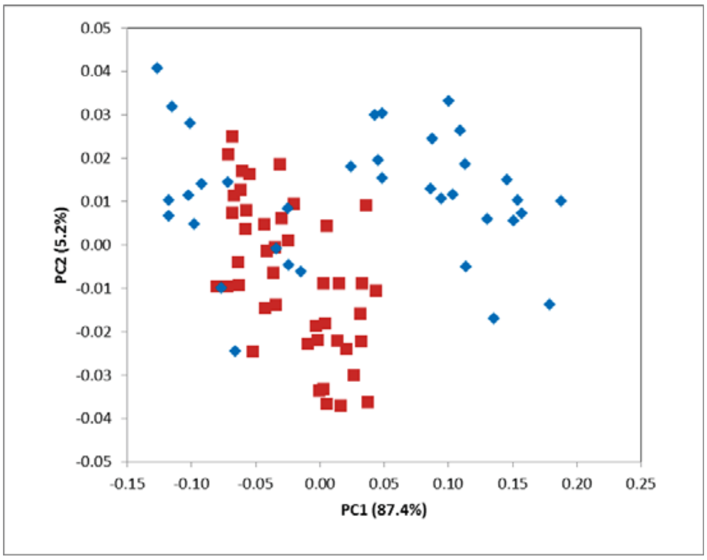


Figure 7.17. Shape variation in ovipositors that are ≥20% of body length. Species with very short ovipositors were excluded. This graph shows that there is more variation in clade 2 (blue) than clade 1 (red) in terms of shape.

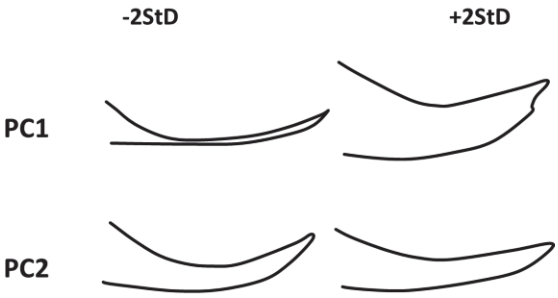


Figure 7.18. Shape changes associated with PC1 (ovipositor breadth) and PC2 (curvature) showing two standard deviations either side of the mean.

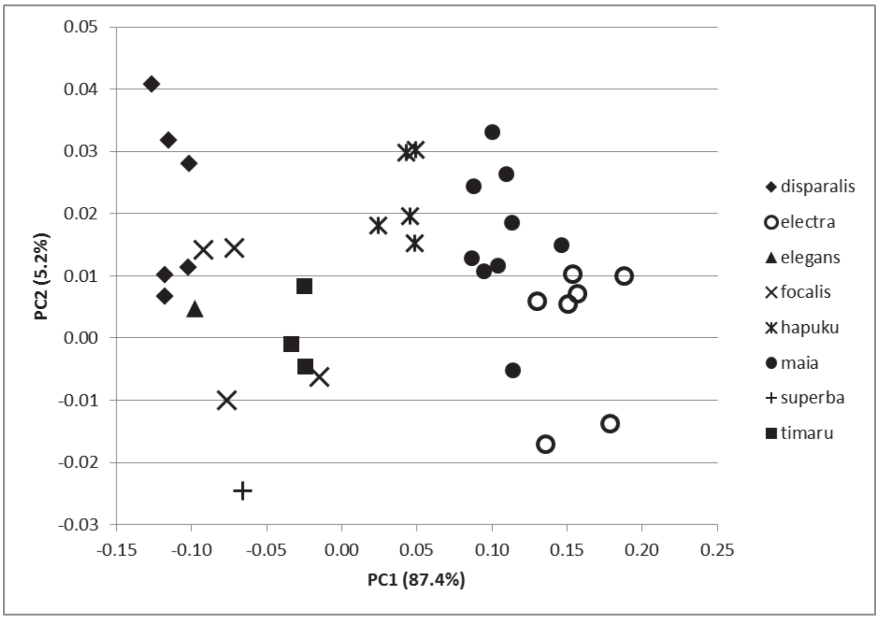


Figure 7.19. PCA replotted from figure 17 to show how shape varies within clade 2. PC1 divides the clade into two with species that have maternal care (*H*. maia, *H. electra* and *H.* “Hapuku”) forming a group.

Discussion

Genetic differences support two clades

Analysis of *Hemiandrus* mitochondrial DNA sequences revealed two distinct clades. Clade 1 included eight described species (*H. subantarcticus, H. fiordensis, H. maculifrons*, *H. nitaweta, H.* ALIUS, *H.* CENTRAL, *H.* NORTH and *H.* PATURAU*)* and one tag-named species (*H.* "madisylvestris") and clade 2 included six described species (*H. electra, H. maia, H. focalis, H. superba, H. bilobatus and H. pallitarsis*) and nine tag-named species (*H.* "hapuku", *H.* "elegans", *H.* "disparalis", *H.* "horomaka", *H.* "vicinus", *H.* "onokis", *H.* "furoviarius", *H.* "kapiti" and *H.* "timaru"). DNA sequences from three nuclear loci supported the relationships inferred from the mtDNA phylogeny, and strengthens the impression that *Hemiandrus* consists of two distinct clades. These have not previously been formally identified and do not correspond with the two groups that Salmon (1950) described as *Hemiandrus* and *Zealandosandrus.*

Morphological differences support two clades

Each of the two main *Hemiandrus* clades had a number of derived morphological traits. They differed in maxillary palp pilosity (Fig. 7.5, 7.6), pronotum shape (Fig. 7.12), superior hind tibial spines and stridulatory pegs (Appendix 7.3). As both clades lack tympana, the secondary loss of which is common in insects (Yager 1999), stridulatory pegs are likely used for defence stridulation (Field 1993) rather than for intraspecific communication. Clade 2 species had more stridulatory pegs than clade 1 species suggesting that defence stridulation may play a more important role in this clade. Clade 1 species, however, had more superior hind tibial spines than variable species, and these may act as alternative defence structures.

I also found that male terminalia (Fig. 7.9) and female sternite modifications (Fig. 7.10) differed between the two clades. Adult females in clade 1 had small pits on the 7th abdominal sternite while those in clade 2 had either pits along the anterior edge of the 6th sternite, or a modified 6th sternite with large lobes (Fig. 7.10). These lobes are part of their mating apparatus with male falci and paraprocts fitting around the lobes or into the pits (pers comm, Darryl Gwynne). Males in clade 1 had sharp, blade-like falci that were usually touching one another and blunt paraprocts (Fig. 7.8, 7.9). Falci of males in clade 2 were usually more widely spaced and their paraprocts had sharpened tips. The reason why male terminalia have evolved to fit female sternites is not certain, but some species with similar male devices use their modifications to force copulations with females when females are unwilling to mate due to a lack of male nuptial gift (Sakaluk *et al.* 1995). In some cases females appear to have evolved structures to thwart male copulation (Arnqvist and Rowe 2002) but sternite modifications in female ground wētā appear to be a sexually selected structures evolved to help females to acquire a nuptial meal

(Gwynne 2005). Sexual selection may cause male-female coevolution in isolated populations and may thus increase speciation rates within clades with high levels of sexual selection. Further evidence for sexual selection comes from the phylogeny showing that *H. pallitarsis* had the most highly modified sternites but its phylogenetic sister, *H.* “Kapiti”, had no lobes but only pits, indicating rapid modification in the organ in allied lineages (Fig. 7.10) .

Of other *Hemiandrus* that have not been formally described, morphological differences between the two clades indicated that *H.* “saxatilis” is likely to belong to clade 1 (pilose MP3 and MP4 (Fig. 7.5) and adjacent male falci (Fig. 7.9)), while *H.* “hunter mountains” (Jewell 2007), which was not examined here, is likely part of clade 2 (MP4 pilose on distal 40%, male falci widely-spaced; Fig. 6 Jewell 2007).

New Zealand ground wētā comprise two genera?

Genera are traditionally phenotypically diagnosable and most genera erected based on morphology alone, “morphogenera”, have been found to be congruent with molecular phylogenies in that they form monophyletic groups (Jablonski and Finarelli 2009). *Hemiandrus* consists of two stable clades each of which has derived characters, therefore *Hemiandrus* can be considered to comprise two genera. The generic name *Hemiandrus* was first applied by Ander (1938) to *H. pallitarsis* (previously *H. furcifer*) and *H. bilobatus*, thus clade 2 should retain this name, while clade 1 should be renamed. The genus name *Zealandosandrus* Salmon should not be resurrected for clade 1 because it was established for long ovipositor species including some belonging to clade 2. These two distinct evolutionary lineages of New Zealand *Hemiandru*s may not be sister clades as suggested by preliminary study of Australian species (Johns 1997; Pratt *et al.* 2008). Inclusion of Anostostomatidae specimens from its global range (Song *et al.* 2015) and ongoing work (Steve Trewick, Eddie Dowle pers comm) will help resolve systematic relationships.

Ecological implications of taxonomic differences

Another criterion that has been suggested for assigning the rank of genus is the occupation of distinctive adaptive zones (Vences *et al.* 2013). An adaptive zone is a set of ecological niches that may be occupied by a group of species exploiting the same resources in a similar manner (Simpson 1944). The niche is the set of biotic and abiotic conditions under which the species is able to persist (Hutchinson 1957). Morphological and behavioural changes associated with resource use and life history can change and/or broaden niches. Species that occupy different niches are expected to fill an area more thoroughly as they can coexist in sympatry while exploiting different resources whereas similar species with large overlap of resource requirements cannot stably coexist in sympatry (limiting similarity) (Darwin 1859; Gause 1934), resulting in competitive exclusion (Hardin 1960).

It may be that ground wētā species have evolved to occupy different niches. In the previous chapter I examined whether tag-names represent distinct species and now I have shown that species fall into two phylogenetically and morphologically distinct clades. Here I explore potential niche differences between clade 1 species and clade 2 species, focusing on ovipositor and egg traits that may influence the range of habitats they can use.

Maternal care

The insect egg has an outer shell, the chorion, consisting of layers secreted by the mother before fertilisation (Zeh *et al.* 1989) (Fig. 7.20). Eggs respire through pores in the chorion (aeropyles) which range in diameter from 0.2 µm to 2.5 µm (Hinton 1981). The water-air interface across the aeropyles acts as a plastron allowing for respiration even when submerged. The efficiency of gas exchange is increased by increasing the number of aeropyles in the chorion, widening their openings, or connecting them (Hinton 1981). These networks also strengthen the chorion thus preventing the egg from breaking due to soil weight upon them or the excessive influx of water. The chorion also contains antimicrobial secretions (Marchini *et al.* 1997). The evolution of adaptive egg surface structures, in conjunction with long ovipositors that can hide eggs from predators and parasites by inserting them into substrates means that eggs of many insect species can survive independently. As a result, insects tend to have low levels of postzygotic parental investment compared to other arthropods (Zeh and Smith 1985).

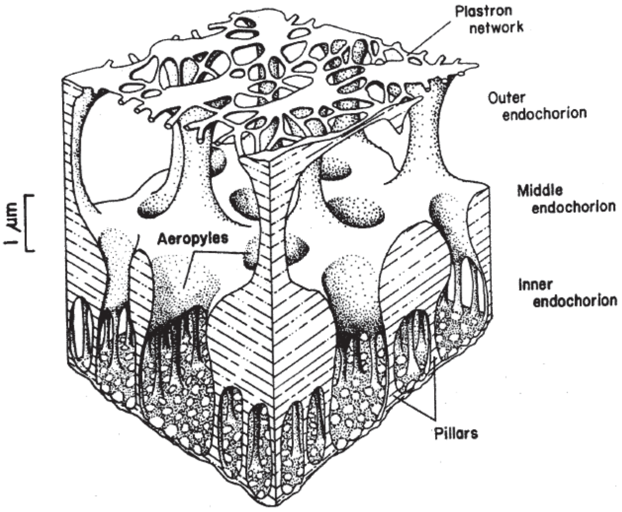


Figure 7.20. In many species of insect, eggs must be able to survive independently of adults in a range of environments. The outermost layer of the egg, the chorion, helps eggs to achieve this. The chorion contains aeropyles, pores through which the embryo respires. The efficiency of gas exchange is increased by the presence of more aeropyles in the chorion, wider openings, and linkage between them. These networks can also increase the strength of the chorion. The water-air interface across the aeropyles acts as a plastron allowing for respiration even when submerged. From Zeh and Zeh 1999.

Long ovipositors and egg abandonment are probably the ancestral condition in Ensifera (Kevan 1989; Rentz 1996), but various maternal care behaviours have been reported in five families including *Hemiandrus* (Anostostomatidae) (Gwynne 1995). These behaviours include guarding eggs and nymphs, palpating and cleaning eggs, patching and sealing burrows and shifting eggs from flooded burrows (Gwynne 2004; Taylor Smith *et al.* 2013). There is no evidence that females feed their nymphs, and it is unlikely given that there is evidence that nymphs leave the burrow to forage and then return (Taylor Smith *et al.* 2013). Care has been observed in various clade 2 species with reduced ovipositors including most species in clade 2a (Gwynne 2004) and some species from clades 2b (*H. maia* (Taylor Smith *et al.* 2013), *H.* “Hapuku” (pers obs), *H. electra* (Steve Trewick, Mary Morgan-Richards, pers comm). All eggs examined of clade 2a species with maternal care and reduced ovipositors had no chorion structures associated with egg independence (Fig. 7.11). The eggs of these species would likely be weak, susceptible to drowning and unlikely to survive unaided. In accordance with this, Wahid (1978) found that the eggs of a very short ovipositor species would frequently pop when handled while Gwynne (2004) found that unaided eggs where susceptible to fungal attacks. The eggs of reduced ovipositor species belonging to clade 2b (*H. electra*, *H. maia*, *H.* “disparalis”, *H. superba*, *H. focalis, H.* “hapuku”) all had some chorion surface structure in the form of many irregularly positioned pores with a maximum diameter of 10-20µm (Fig. 7.11). These species had longer ovipositors than egg-caring species in clade 2a and their ovipositors were similar in length to some clade 1 species. Although there is not necessarily a length difference between care and no care species, there appears to be a difference in ovipositor shape (Fig. 7.19).

Egg abandonment, although assumed to be the norm, has only be observed in two long ovipositor species: *H. subantarcticus* (Butts 1983) and *H. focalis* (Gwynne 2004). However, few observations of ground wētā behaviour have been published due to their size and nocturnal nature. Another long ovipositor species, *H. maculifrons*, has been observed to oviposit into the walls of her gallery (Cary 1981). Eggs of this species have been found in an advanced state suggesting that the same female had remained in the gallery for months, but there have been no records of the presence of nymphs in a female’s burrow and first instar nymphs have been found in burrows of their own (Cary 1981). Therefore it is unclear whether this is an example of maternal care, an intermediate evolutionary step or simply opportunistic oviposition.

Morphological traits are not able to vary freely. Rather, developmental processes are a source of evolutionary constraint (Maynard Smith *et al.* 1985) with some changes in the developmental process easier than others. If taxa are similar and they share the same developmental processes then they are more likely to evolve in the same way i.e. convergent evolution. When traits evolve convergently, it does not necessary mean that it is an adaptation that has been selected for; convergent evolution can also be a coincidence, an exaptation or a correlated response to selection on another character (Losos 2011). It is clear that maternal care has evolved convergently in *Hemiandrus*. The behaviour of *H. maculifrons* may be an intermediate step between egg abandonment and the care and defence of eggs and nymphs, and highlights the possibility that care has evolved independently in *Hemiandrus* species as an exaptation of living and ovipositing in the same substrate rather than because it is a beneficial adaptation. Genera such as tree wētā (*Hemideina*) would be much less likely to evolve care because they live in trees but oviposit in soil.

I have shown that in some ground wētā species, a reduction in ovipositor length is concordant with a loss of adaptive chorionic structures. This is commonly seen in insect species which care for their eggs (Hinton 1981; Zeh *et al.* 1989), probably because these adaptations are structurally and energetically expensive to maintain. However, some caring species have somewhat reduced ovipositors but appear to have retained adaptive chorionic structure. A possible explanation is that morphology differs between caring species due to the presence of different degrees or forms of care. However, I think that it is more likely that maternal care has evolved more recently in these lineages with insufficient time for the loss of structures associated with independent eggs. As well as evolving de novo in *Hemiandrus*, it is possible that maternal care may be reversible as long as adaptive structures associated with the independent survival of eggs are retained. However, if all adaptive structures are lost, as in the case of some species in clade 2a which have almost no ovipositor or chorion adaptations, then species may be trapped by phylogenetic inertia.

Distribution

Clade 1 *Hemiandrus* species were found only in wet native forests or mountain ranges with high rainfall and were absent from secondary native forest fragments. On the other hand clade 2 species were found in a wide variety of environments, including hot and dry areas, primary and secondary forests, and highly modified environments such as exotic forest, farmland, orchards and vineyards. On South Island this pattern was quite striking with many clade 2a species occupying the east coast, a highly modified region which has low rainfall, high temperatures and long sunshine hours resulting in a water deficit and thus relatively dry soil (Fig. 7.21). No clade 1 species were found in this region, whereas Westland and Fiordland on the west coast of the island had many clade 1 species as well as long ovipositor 2b species but no clade 2a species, nor any species with, or suspected to have maternal care. In fact, species with maternal care were found throughout the country and appeared to be absent only from Fiordland and Westland, regions which have very high rainfall (Fig. 7.21).

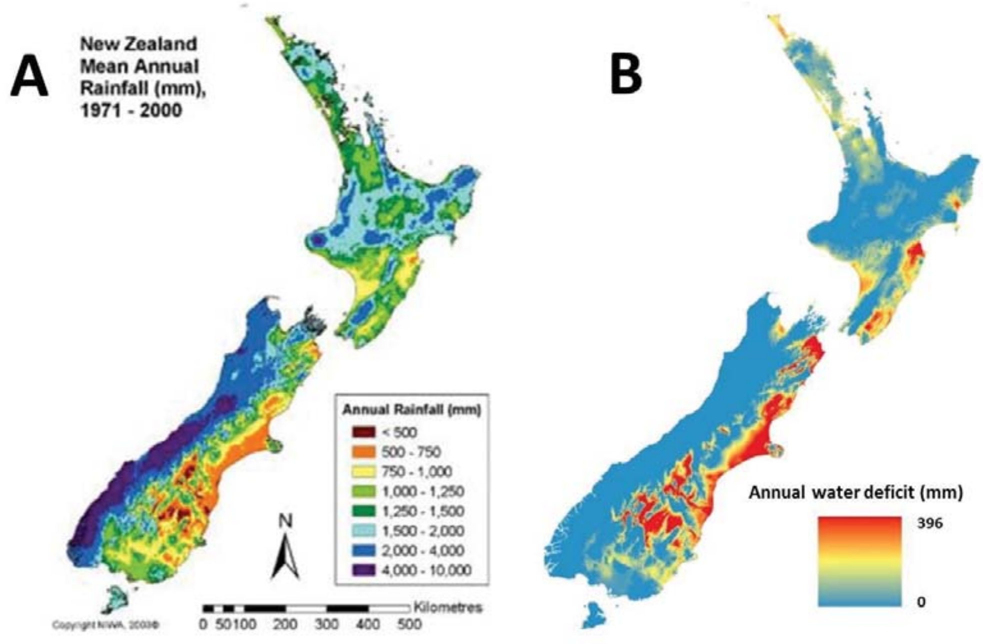


Figure 7.21. Mean annual rainfall and water deficit in New Zealand. No species with maternal care are found in western South Island where rainfall is high (**A**). Species with minute ovipositors are found throughout the very dry regions of eastern South Island (**B**).

Although these differences in distribution may be the result of stochastic processes or competition, they can also be explained by differences in morphology and behaviour of ground wētā species: the ability of species with long ovipositors to inhabit very wet regions is likely due to the fact that all species in this clade have retained chorionic adaptations associated with strength and underwater respiration. Large aeropyles allow eggs to respire in wet conditions and to withstand damage caused by soil movement. The ridges on some of the eggs in clade 1 and on *H.* "disparalis" eggs (Fig. 7.11) likely also strengthen the egg and may help water to drain from the egg. Eggs with no adaptations would likely drown or be crushed due to soil movement, but even if eggs do have adaptations to withstand some of these forces, care may be maladaptive in wet environments because the energetic costs of patching burrows or shifting eggs may exceed the benefits of care. So although maternal care has likely helped the survival of individuals and species through environmental disturbance and changes, it is probably not the best life history strategy in all environments.

Genera are traditionally phenotypically diagnosable but they may also represent species with distinct ecological requirements. Clade 1 species of *Hemiandrus* appear to be adapted to wet native forest and mountain habitats and have eggs and ovipositors adapted to egg abandonment while clade 2 species have a variety of life history strategies which allow them to survive in a variety of habitats. Nevertheless, the niche variation within *Hemiandrus* helps to explain why there are so many species of ground wētā compared to other NZ anostostomatid genera.

Conclusion

Hemiandrus is a genus with high species diversity relative to other New Zealand anostostomatid genera. Concordance of nuclear markers with mtDNA confirms two clades. The species within each of the two clades share derived morphological traits including distinct differences in the morphology of their mating apparatus. The two clades also appear to have differences in life history and ecology. This suggests that *Hemiandrus* comprises two genera and that the high species diversity is the result of two genera undergoing separate radiations. Diversification and the retention of diversity within these two clades may also have been promoted by various species traits. For example, *Hemiandrus* ground wētā have various traits that indicate they are or have been subject to strong sexual selection, the most obvious of which are nuptial gifts and female sternite modifications for receiving nuptial gifts (Gwynne 2004, 2005). Sexual selection on traits that are involved in mating can cause the rapid divergence of traits and reduced interbreeding between incipient species (Civetta and Singh 1998) resulting in a significant positive correlation between sexual selection and speciation rate (Kraaijeveld *et al.* 2011). Therefore the high species diversity within *Hemiandrus* may have been driven by high levels of sexual selection. Furthermore, some clade 2 species have maternal care, a trait which has not been reported in any other Anostostomatidae. This behaviour is correlated with a reduction in ovipositor length and involves the female remaining with and guarding the eggs and nymphs in her burrow. This may allow species with maternal care to survive through fluctuating climatic conditions or habitat changes, although care may be maladaptive in wet habitats.

Appendix

Appendix 7.1. Primers used to amplify and sequence DNA from *Hemiandrus*, plus the number of *Hemiandrus* individuals sequenced for each locus and the model used.

Locus	Name	Primer 5’ to 3’ sequence	Length (bp)	Model	n	Outgroup
Nu2	Nu2Hemi1212F	GAGGCYTGCGT ACAAAAACG	209	K80	53	No
	Nu2Hemi1545R	GCTGATGATCC AGGCGGTAA				
Nu3	Nu3Hemi680F	GCGTCATCCCA GCTCTTCTT	217	TRN+I	49	No
	Nu3Hemi989R	GACCATCTATGC CTCGGACG				
Nu13	Nu13pall2078F	CGGAAGTGCTC CCAATCAGT	194	HKY+I	43	*Hemideina spp*
	Nu13pall2375R	GGGGCTCGAGG CTATCTCTA				

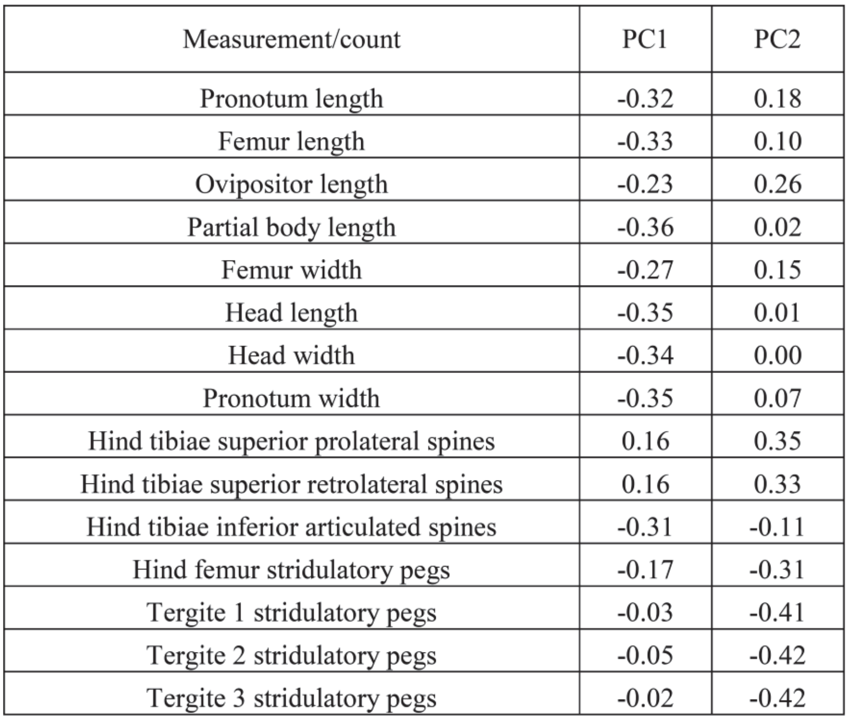
Appendix 7.2. Female body measurements. Many adult females did not contain eggs. Also, some measurements were not able to be recorded for some species (NR).

Species			PL	FL	OV	Total BL	BL	FW	HL	HW	PW	mean egg length
Clade 2	*bilobatus* (n=8)	mean	5.02	11.88	2.15	19.26	12.18	3.48	8.34	5.91	5.77	3.36
		sd	0.26	0.62	0.30	2.31	1.46	0.24	0.62	0.45	0.55	NA (n=1)
	"horomaka" (n=6)	mean	4.51	11.17	1.50	20.14	9.91	3.14	7.41	4.98	5.04	NA
		sd	0.52	0.85	0.22	0.70	0.81	0.40	0.64	0.55	0.71	NA
	"kapiti" (n=1)		5.40	13.92	NR	NR	12.42	4.16	8.42	5.66	6.29	NA
	"onokis" (n=3)	mean	4.35	10.14	2.04	16.58	9.50	3.10	7.32	5.02	4.86	3.53
		sd	0.28	1.04	0.28	3.05	1.43	0.30	0.51	0.56	0.40	NA
	pallitarsis (n=5)	mean	4.81	12.28	1.94	20.11	10.68	3.52	7.79	5.26	5.48	3.44
		sd	0.32	0.89	0.33	1.54	0.46	0.19	0.42	0.24	0.23	0.33
	"timaru" (n=3)	mean	4.63	12.70	10.61	17.12	10.41	3.76	6.86	4.89	5.56	3.41
		sd	0.43	0.20	0.24	2.14	0.70	0.28	0.22	0.03	0.08	0.08
	"vicinus" (n=4)	mean	4.91	12.25	2.23	20.85	10.95	3.76	7.85	5.43	5.54	3.17
		sd	0.46	0.92	0.13	3.09	0.87	0.17	0.70	0.42	0.38	0.34
	"disparalis" (n=7)	mean	5.54	18.09	18.60	19.74	11.98	4.10	7.86	5.05	6.28	3.90
		sd	0.32	0.90	0.32	3.00	0.90	0.22	0.23	0.22	0.32	0.25
	electra (n=11)	mean	5.80	16.10	6.11	20.22	12.01	4.29	8.45	5.90	6.12	4.00
		sd	0.28	0.95	0.69	1.02	0.90	0.26	0.59	0.47	0.38	0.21
	"elegans" (n=1)		6.72	22.44	19.08	24.50	14.66	5.25	9.59	6.39	7.69	NA
	superba (n=1)		7.92	21.62	21.5	36.2	20.18	4.75	15.5	12.58	11.75	5.20
	"hapuku" (n=4)	mean	6.04	17.15	10.58	25.06	14.51	4.32	10.90	7.59	7.80	4.48
		sd	0.30	0.44	0.29	4.64	1.76	0.17	0.16	0.28	0.27	0.33
	focalis (n=3)	mean	6.49	15.49	16.19	24.75	14.39	3.70	11.34	8.49	8.32	4.87
		sd	0.39	0.66	1.70	2.96	1.42	0.10	0.81	1.01	0.80	0.15
	maia (n=13)	mean	5.69	16.07	7.86	22.86	12.47	4.29	9.49	6.50	6.73	4.24
		sd	0.43	1.66	0.61	1.88	0.85	0.32	0.62	0.35	0.36	0.20
Clade 1	ALIUS (n=26)	mean	4.76	11.16	7.63	12.69	7.95	3.49	5.54	3.55	4.56	2.80
		sd	0.23	0.72	0.74	1.87	0.72	0.27	0.49	0.19	0.36	0.08
	fiordensis (n=4)	mean	6.76	17.21	15.52	26.19	14.98	4.07	9.88	6.35	7.23	4.32
		sd	1.11	2.34	1.85	3.01	2.09	0.51	1.21	0.70	0.93	0.26
	NORTH (n=81)	mean	4.76	12.03	9.44	13.24	8.54	3.70	5.77	3.96	4.93	2.75
		sd	0.44	0.96	0.84	2.41	0.95	0.33	0.46	0.29	0.38	0.13
	CENTRAL (n=34)	mean	4.60	11.48	9.35	13.91	8.42	3.41	6.01	4.05	4.54	2.83
		sd	0.31	0.55	0.57	1.20	0.75	0.31	0.24	0.19	0.34	0.36
	"madisylvestris" (n=3)	mean	6.04	15.52	13.04	19.84	11.36	4.07	8.51	5.52	6.21	3.68
		sd	0.13	0.59	0.10	2.51	0.02	0.19	0.46	0.10	0.39	0.18
	nitaweta (n=4)	mean	5.97	14.21	15.42	25.08	13.60	3.77	9.80	6.54	6.81	4.32
		sd	0.24	0.82	1.25	2.45	1.32	0.18	0.49	0.25	0.19	0.04
	PATURAU (n=1)		4.31	11.69	10.21	13.00	7.93	3.24	5.79	3.90	4.41	2.90
	maculifrons (n=63)	mean	4.58	12.79	12.03	15.31	8.91	3.46	6.11	4.10	4.61	2.89
		sd	0.43	1.35	1.32	1.61	0.85	0.31	0.39	0.31	0.41	0.32
	subantarcticus (n=2)	mean	8.20	17.05	14.12	26.03	14.32	5.52	10.39	7.14	8.36	3.66
		sd	0.54	0.99	1.55	2.27	1.56	0.37	0.86	0.46	0.73	0.56

Appendix 7.3. Spine and peg counts

species			P spines	R spines	Mean hind tibial spines	Leg pegs	Pegs T1	Pegs T2	Pegs T3
Clade 2	*bilobatus* (n=5)	mean	7	8	3	92	>100	>100	>100
		sd	0.42	0.32	0.27	57.96	NA	NA	NA
	"horomaka" (n=3)	mean	6	8	3	73	77	>100	70
		sd	0.29	0.50	1.26	9.45	15.28	NA	14.14
	"kapiti" (n=1)		7	9	1	NR	NR	NR	NR
	"onokis" (n=3)	mean	7	8	4	54	57	>100	>100
		sd	0.29	0.50	0.87	0.71	20.03	NA	NA
	pallitarsis (n=5)	mean	8	8	3	87	>100	>100	>100
		sd	0.55	0.65	0.76	29.79	NA	NA	NA
	"timaru" (n=3)	mean	8	8	3	43	65	70	68
		sd	0.00	0.00	0.58	13.32	21.21	14.14	28.43
	"vicinus" (n=4)	mean	7	8	3	86	73	90	90
		sd	0.58	0.58	0.58	9.07	15.28	0.00	0.00
	"disparalis" (n=3)	mean	9	8	6	75	61	87	68
		sd	0.29	0.00	1.00	3.46	8.08	11.55	7.64
	electra (n=11)	mean	8	8	3	54	>100	>100	>100
		sd	0.48	0.50	0.48	7.54	NA	NA	NA
	"elegans" (n=1)		8	8	8	48	50	80	60
	superba (n=1)		7	8	6	80	50	60	50
	"hapuku" (n=4)	mean	8	9	7	198	>100	>100	>100
		sd	0.29	0.50	0.87	24.58	NA	NA	NA
	focalis (n=3)	mean	7	8	6	75	39	52	42
		sd	1.00	0.29	0.58	25.42	14.93	23.63	20.21
	maia (n=13)	mean	8	8	4	33	60	70	45
		sd	0.64	0.70	0.64	4.93	14.14	14.14	21.21
Clade 1	ALIUS (n=26)	mean	14	12	0	0	21	21	21
		sd	0.61	0.98	0.00	0.00	4.24	2.83	0.00
	fiordensis (n=4)	mean	11	10	4	47	14	21	22
		sd	1.89	2.36	0.87	9.64	6.24	3.61	3.51
	NORTH (n=69)	mean	15	15	1	7	46	49	41
		sd	1.64	1.63	0.30	12.49	13.99	11.49	11.46
	CENTRAL (n=24)	mean	13	12	1	22	33	43	38
		sd	1.38	1.48	0.22	12.34	9.50	9.68	10.07
	"madisylvestris" (n=3)	mean	17	15	4	31	8	14	19
		sd	0.71	1.06	0.58	1.41	2.83	2.12	3.54
	nitaweta (n=4)	mean	7	7	3	42	9	20	24
		sd	0.00	0.91	0.85	7.59	2.71	9.56	6.22
	PATURAU (n=1)		15	15	1	15	20	15	20
	maculifrons (n=56)	mean	14	13	2	31	31	41	38
		sd	1.35	1.45	0.30	14.32	10.11	11.13	10.54
	subantarcticus (n=2)	mean	15	15	3	0	4	8	2
		sd	0.71	0.71	0.71	0.00	0.00	7.78	0.71

Appendix 7.4. PCA loadings



Measurement/count	PC1	PC2
Pronotum length	-0.32	0.18
Femur length	-0.33	0.10
Ovipositor length	-0.23	0.26
Partial body length	-0.36	0.02
Femur width	-0.27	0.15
Head length	-0.35	0.01
Head width	-0.34	0.00
Pronotum width	-0.35	0.07
Hind tibiae superior prolateral spines	0.16	0.35
Hind tibiae superior retrolateral spines	0.16	0.33
Hind tibiae inferior articulated spines	-0.31	-0.11
Hind femur stridulatory pegs	-0.17	-0.31
Tergite 1 stridulatory pegs	-0.03	-0.41
Tergite 2 stridulatory pegs	-0.05	-0.42
Tergite 3 stridulatory pegs	-0.02	-0.42

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Chapter 8. All animals are equal, but some animals are more equal than others (Orwell).

“Species” are not equal units

Taxonomy is the practice of classifying and naming biodiversity. Thus, the purpose of taxonomy is to deepen our understanding of the organisms themselves by determining their evolutionary relationships to one another and to develop a common language so that people know what others are talking about when referring to a particular organism. The assignment of names to organisms is based on hypotheses of species boundaries which are thus falsifiable. The process of determining the placement of species boundaries is referred to as “species delimitation” and is based on this idea of discontinuation in character variation between species.

Some believe that the goal of taxonomy should be to name and identify every single living organism in order to create an inventory of biodiversity (e.g. “New Zealand Inventory of Biodiversity”, Gordon 2010). In theory an inventory of biodiversity might be possible, although in reality it is highly unlikely that a final list would or could ever be created. The main problem is that, although, to a degree diversity may exist in units (i.e. species), these units are not all equal and cannot be measured as such. Different species and groups of species have arisen under different evolutionary scenarios and so have different species-associated properties (de Queiroz 2007). Therefore, species delimitation is not a one-size-fits-all analysis and the number of species delimited depends on the criteria being applied. Boundaries in some taxa may be fairly obvious but most groups require intense study in order to formulate and test species hypotheses. For example, my analysis of the *H. maculifrons* species complex showed that two genetic lineages, (*H.* NORTH[8] and *H.* CENTRAL) had males with distinct terminalia even in sympatry. In many insects, male genitalia diverges rapidly due to sexual selection (Eberhard 1985), and such differences can be prezygotic barriers and so can be used for species identification. However, I found within a single *H.* NORTH population two distinct male terminalia morphs that were discordant with other lines of evidence so were unlikely to be associated with a species boundary. *Hemiandrus H.* CENTRAL and *H. maculifrons* (sensu South lineage, see chapter 3) were genetically differentiated and were found in sympatry where they differed in ovipositor length and spine number. Observations of additional individuals from

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[8] Species described in chapter 4 are treated here as described species but species names are given in capitals to distinguish them from formally described species.

allopatric sites (chapter 4) showed that these putative species sometimes have the same number of spines. There were no obvious differences in male terminalia between these two species, yet I found no evidence of gene flow between them. If I had approached the *H. maculifrons* species complex taxonomy problem using only morphological data or if sampling had not included the zone of sympatry at Lewis Pass, it is possible that I would have concluded the analysis with different hypotheses of species boundaries. This highlights the importance of good sampling and the use of multiple types of evidence (i.e. a combination of genetic, morphometric, distributional and ecological data) in the establishment of species boundaries. However, since each species has arisen under a different evolutionary scenario it is not possible to know how much data is required for a given system until a new line of data disagrees with the established hypotheses. It is therefore inevitable that under our current practice of "iterative taxonomy" (Yeates *et al.* 2011), the addition of more evidence will, in many cases, require modification of accepted species boundaries. Therefore a worldwide inventory of biodiversity is likely only ever to be a working hypothesis.

In recent years, with developments in both theory and data availability, many new methods for species delimitation have been developed. Although some of these tools are useful for examining some of the expected attributes of species (e.g. for examining the clustering of sequence data), many are based on too many assumptions about what a species is to be of much use. For example, P(Randomly distinct) (Rodrigo *et al.* 2008), which is a phenetic test of genetic distinctiveness, is based on a coalescent model where the species is panmictic and of constant size. This is unlikely to be the case for many species, if any. These methods therefore are not testing for species boundaries, but for patterns that sometimes exist at some known species boundaries. A clear example of a species delimitation method that cannot be used to determine the position of species boundaries in all scenarios is illustrated by my work on *Hemiandrus* using the Gaps in Continuous Characters across Geography (GCCG) method (Zapata and Jimenez 2012). This attempts to delimit species by detecting gaps in multivariate data with the assumption that for a particular morphological trait or suite of traits, an absence of gene flow results in a difference in central tendency between species traits (i.e. a difference in species means) and a gap in character variation between species (assuming that phenotype is not plastic). If a gap is detected in the data, GCCG assesses whether the gap in morphology is better explained by geographic variation or a species boundary. Of the 14 research articles that have cited this paper, most mentioned it only as an example of a morphology-based species delimitation method and none applied it to a new dataset. In chapter 3 I found differences in relative ovipositor length of females and relative subgenital plate length of males between some lineages. Both of these differences were visible to the naked eye, in part because they were also associated with shape differences. This obvious difference was not large enough to be considered a significant gap by GCCG, which suggests that this method may only be sensitive to extreme gaps. If this is the case, the approach is no better than the naked eye. Furthermore, some "good" species may have no obvious morphological gaps or may have gaps that are not concordant between different characters. The absence of gaps, or discordances among different lines of evidence should be given an evolutionary explanation, and may lead to the use of only some characters for species delimitation (Padial and De La Riva 2010).

My research has led me to agree that species delimitation analyses should have a goal in mind (Carstens *et al.* 2013). Having a purpose for species delimitation influences the choice of methods used, for example "studies that seek to conduct taxonomic revisions and describe new species are less justified in favouring the results of a method . . . that tends to delimit a larger number of lineages than studies that seek to use the delimited lineages in downstream ecological analyses" (Carstens *et al.* 2013). This highlights again the fact that species are not absolute and equal units and clearly illustrates that methods that are being labelled as "species delimitation" methods are nothing of the sort and that the unit that is being measured varies between analyses. The failure of "species delimitation" methods suggests that there are no shortcuts to delimiting species boundaries. Taxonomists should therefore shift away from treating "species" as something objective that can be inventoried and move their focus towards understanding the continuum of biodiversity in its evolutionary context.

Microendemism and species ranges in New Zealand

Widespread species of New Zealand invertebrate are few. This is exemplified by ground wētā with most species being highly range restricted (Jewell 2007; Johns 2001). Although I found many *Hemiandrus* species are more widespread than previously recognised, most do have relatively small ranges. *Hemiandrus* species with the largest ranges occupied less than 40% (approximately 100,000km^2) of the total land area of New Zealand, while species with narrowest ranges occupied less than 20km^2. The number of endemic species within a New Zealand region (average region size 9500km^2) is positively influenced by the total species diversity and land area of that region 3 million years ago. It is likely that the process of speciation can divide a single range into multiple species, each occupying part of the ancestral range. Factors controlling subsequent range expansion (competition, ecology and adjacent land) will interact in a complex way (Darwin 1859; Holt 2003; MacArthur 1972; Sexton *et al.* 2009). Thus a focus on speciation processes and recent range shifts might inform on current patterns of endemism (Nattier *et al.* 2012).

Prior to my work, only a single ground wētā species (*H. maculifrons*) had been reported extensively throughout both main islands of New Zealand. The genetic and morphological evidence presented in this thesis revealed that this unusually widespread taxon is better treated as a species complex comprising multiple species with narrower parapatric ranges. This illustrates that a region can be filled by a single species or by many similar species with restricted ranges. The relationships between the species involved in this example may reveal the drivers of regional microendemism. Both patterns (i.e. a single species filling a large area or an area being filled by many closely related range restricted species) are present within the *Hemiandrus* clade of eight species found on North Island and the east coast of South Island (*H. pallitarsis*, *H. bilobatus*, *H.* "kapiti", *H.* "vicinus", *H.* "onokis", *H.* "horomaka", *H.* "furoviarius", *H.* "timaru"). *Hemiandrus pallitarsis* fills most of the available area on North Island including the geologically young regions. Only one other species in this clade, *H. bilobatus*, is present on North Island where it is restricted to Wellington but is also found on South Island. *Hemiandrus* "kapiti" is found on Kapiti Island near Wellington but is not present on North Island. In contrast, six species belonging to this clade are found on the east coast of South Island where they have adjacent but largely non-overlapping ranges suggesting that speciation has subdivided ancestral ranges. The geographical signal of speciation decays with time as the result of changes in species ranges (Losos and Glor 2003), but the presence of sister species with adjacent ranges indicates that range changes have not erased the geographic signal of speciation. Therefore this system may be useful for inferring speciation processes and may help to reveal universal drivers of microendemism.

Closely related to these east coast microendemics is *H.* "kapiti" which is endemic to Kapiti Island. The Kapiti Island ground wētā is sister to *H. pallitarsis* which is found throughout North Island. These two species are well-diverged with clear morphological differences in male terminalia, female sternite modifications and mid tibial spines number. Phylogenetic analysis indicates that these two species diverged earlier than some other well-supported sister taxa. The most obvious cause of speciation between *H. pallitarsis* and *H.* "kapiti" is allopatry with *H.* "kapiti" evolving in isolation on Kapiti Island separated from North Island by the Rauoterangi Channel. This scenario is, however, unlikely because Kapiti Island is geologically part of North Island (Moore and Francis 1988) and land did not exist in the position of Kapiti Island even 1 million years ago (Trewick and Bland 2011). As recently as the Last Glacial Maximum and earlier glacial periods, lowered sea levels resulted in the two islands being connected (Alloway *et al.* 2007). Perhaps then, *H.* "kapiti" was once much more widespread, and became extinct from other regions, surviving only on Kapiti Island. Extirpation may have been the result of interspecific competition with *H. pallitarsis* (or with another ground wētā species) and if there was no ocean barrier surrounding the range of *H.* “kapiti”, perhaps competition with *H. pallitarsis* would have resulted in extinction of the whole species.

In chapter 1 I found that patterns of diversity and endemism are influenced by both ancient and recent processes including past available terrestrial habitat, degree of isolation and climate. The biggest effector of the relationship between endemism and land area 3Ma was the former absence of land in southern North Island. This is a clear instance of history (tectonics) having a greater influence on species distributions than current ecology. Within ground wētā, there is evidence that species distributions are influenced by interspecific competition, climate, land availability and connectivity of regions. Furthermore they may be affected by life-history traits.

It has been shown that range sizes of some taxa correlate with estimated time since their origin (Nattier *et al.* 2012), with species expanding their ranges early on before declining to extinction (Foote *et al.* 2007). The ranges of some ground wētā show that range size also depends on geometric constraint and the location of congenerics. *Hemiandrus pallitarsis* has a very large range compared to its close relatives, and it may have expanded its range in a south to north direction to fill much of North Island as it emerged from the ocean. Evidence for the ability of *H. pallitarsis* to expand into new habitat comes from its tolerance to habitat modification; unlike long ovipositor species that are absent from modified land, *H. pallitarsis* persists in parks and gardens. The ranges of is close relatives may have been geometrically constrained by available habitat and interspecific competition while that of *H. pallitarsis* may not have been. This illustrates that although species’ range size is subject to phylogenetic constraints (Abellán and Ribera 2011; Jablonski 2000), even closely related species can differ in the size of their range.

Species ranges are also influenced by interactions with other species; Darwin (1859) observed that “the struggle for existence” is stronger between more closely related species and concluded that this was because close relatives are more likely to occupy similar niches. Mutual interactions between closely related taxa inhibit each other’s ability to expand their ranges and secondary sympatry can take millions of years following divergence (Weir and Price 2011). Species that are more phylogenetically and morphologically diverged are more likely to have reached the stage of secondary sympatry (Pigot and Tobias 2013). Most long ovipositor species appear to be unevenly distributed and have large overlap in their ranges although the parapatric distributions of some long ovipositor species imply that some close relatives do not overlap and may experience competition. On North Island where *H.* CENTRAL and *H.* NORTH meet along the axial ranges (chapter 3) and on Mt Taranaki (chapter 4), *H.* CENTRAL is always found at higher altitudes than *H.* NORTH suggesting that *H.* CENTRAL may be adapted to cooler habitats and its range is the result of an interaction between the environment and interspecific competition. This mimics the patterns seen in two tree wētā species (Bulgarella *et al.* 2014; Trewick and Morgan-Richards 1995). Life history traits also impact species ranges (Dennis *et al.* 2005; Gaston *et al.* 1997), and may play a role in shaping the distribution of *Hemiandrus* species: except for *H. pallitarsis*, species with maternal care appear to have small ranges with little overlap through most of New Zealand. This is most obvious in northwest South Island which is occupied by many long ovipositor species, but only a single species with maternal care.

Maternal behaviour

Insects have morphological adaptations that allow eggs to be abandoned and survive independently. Because of this, parental care of eggs and nymphs is uncommon. Insects with maternal care tend to have shortened ovipositors and eggs lacking chorionic structures associated with independence (Hinton 1981; Zeh *et al.* 1989). Although a reduction in ovipositor length is associated with care in *Hemiandrus*, it appears that the correlation is not perfect. The retention of egg modifications and the longer ovipositors in some species may mean that in *Hemiandrus* care is an evolutionary plastic trait (Lin *et al.* 2004) and that care of offspring may be favoured in some scenarios, but not all. Variation can exist in ovipositor length due to factors other than care. Ovipositor length in species without maternal care may depend on a trade-off between the predation risk and structural costs to the mother, the benefit of deep insertion, the ability of hatchlings to reach the surface and female oviposition behaviour. Cricket hatchlings from more deeply laid eggs are less likely to reach the soil surface, but survival is more likely for larger hatchlings (which come from larger eggs) (Bradford *et al.* 1993). In crickets, oviposition depth is correlated with ovipositor length but females can lay at a variety of depths in response to climate (Bradford *et al.* 1993) and soil conditions (Herrmann *et al.* 2010). A preliminary investigation into the relationship between ovipositor length and egg size indicated that egg size is more strongly correlated with female body size rather than ovipositor length.

The evolution of maternal care depends on ecological constraints and opportunities, the capacity of the female to increase the survival of her offspring and the ability of offspring to survive without care (Tallamy and Brown 1999). Parental care is usually modelled in terms of costs and benefits. Possible advantages of maternal care include decreased predation and energetic costs due to having a lower maintenance and less cumbersome short ovipositor, increased ability for eggs to survive through environmental disturbance that may for example, benefit species in range expansion (as discussed above) and increased survivorship of offspring due to care. There is, however, no good evidence that within Insecta maternal care of offspring is better at reducing predation and other causes of mortality in offspring than reproduction without care (Tallamy and Brown 1999). Possible disadvantages of care include lost mating opportunities (but see Trumbo (2013)) and the risk to offspring being condensed in space and time.

The convergent evolution of care in *Hemiandrus* may not be because it is beneficial, but rather because there may be few costs to caring. The presence of care in some groups is likely constrained by phylogeny, season and/or resources so that there is little fitness gain in doing so (Tallamy and Brown 1999). For example, the horsefly *Goniops chrysacoma* guards its eggs not because they have a greater predation risk than eggs of other species but because the female cannot lay again until she gets a bloodmeal from a vertebrate (Pechuman 1981). The processes leading to the evolution of care can be difficult to elucidate because it is difficult to distinguish between drivers and consequences of maternal care. For example, maternal care may drive sexual selection for high paternal investment in the form of nuptial gifts, which may then lead to polyandry but polyandry results in a low level of offspring relatedness which can drive the evolution of care (Bonsall and Klug 2011).

Some insects have a caring strategy that involves laying away from the nest (e.g. burying beetles, Pukowski (1933)) and in these species, egg-independence structures are retained (Royle *et al.* 2012). The behaviour of *H. maculifrons*, which has retained a long ovipositor and egg chorionic modifications, yet oviposits in the walls of its burrow, may be an example of maternal care. This behaviour can also be viewed as an intermediate step between care of nymphs and egg abandonment. Parental care is common in ground-burrowing arthropods (Nardi 2009) and most ensiferans with maternal care are ground-burrowers (Gwynne 1995). This relationship has been attributed to the susceptibility of eggs to fungal attack in a humid environment (Boos *et al.* 2014). However, care may evolve readily in ground-burrowing arthropods also because adults and eggs are concealed in the same substrate and may explain why care is present in many ground wētā species but no other New Zealand anostostomatids.

Conclusion

Phylogeography uses the geographic distribution of genetic variation to interpret the role of historical processes in the development of biological distributions and deals primarily with the structuring of populations within species (Avise 2000). This focus distinguishes it from phylogenetics and the use of species-level phylogenies to infer biogeography (Arbogast and Kenagy 2001; Brooks 1985; Cracraft 1994; Croizat *et al.* 1974; Nelson and Rosen 1981). However, many of the pitfalls of biogeographic interpretation from species trees apply also to intraspecific phylogeography – there being a natural evolutionary continuum underpinning population genetics, intraspecific phylogeography, interspecific phylogeography and species phylogeny (Page 1994). This has led to a call for the use of interspecific phylogeography rather than reliance on a single species (Trewick *et al.* 2011). Traditionally, New Zealand phylogeographic studies have focused on single widespread species based on the idea that their wide range will likely encompass features of interest that might have impacted on their spatial patterns of genetic diversity. My research shows that it is only when species are considered in context of their life history traits, their environment and their close relatives that we can make inferences about past processes. Furthermore my research shows that widespread species are uncommon. This is likely because most species are unable to maintain gene flow between geographically distant populations and so populations may diverge to become separate species before a large range can be attained. Widespread species tend to have higher connectedness and therefore higher levels of gene flow than species with narrower ranges (Slatkin 1987). Widespread species therefore give a biased interpretation of biogeographic processes.

My work illustrates the fundamental role that taxonomy and phylogenetics have in understanding historical process, ecological interactions, conservation and future outcomes of habitat modification: once you have an understanding of species boundaries, ranges and relationships, more profound questions can be posed and explored. Much research is based on the assumption that species boundaries exist without those limits ever being tested. *Hemiandrus maculifrons* for example was erected by Walker in 1869 on the basis of a single individual, but had never been examined. Even species boundaries that have been explored more thoroughly should not be taken as absolute entities.

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