Speciation and phylogeography in the New Zealand archipelago

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„There is grandeur in this view of life, ... that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved."

Charles Darwin - *The origin of species by means of natural selection* - 1859

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Introduction

Structure and scope of the thesis

This thesis consists of five self-contained chapters, each focusing on questions about the assemblage, biogeographic structuring and evolutionary history of elements of New Zealand's fauna.

The first of these chapters (II) summarizes the current state of knowledge regarding the evolution of the terrestrial fauna of New Zealand based on molecular data and considers the quality of alternative evidence (published in *Philosophical Transaction of the Royal Society B*). The following chapter (IIIa& b) examines the radiation and evolutionary history of an endemic carabid beetle genus (*Mecodema*). Within this chapter, I use molecular dating to study rates of evolution in invertebrates and the correlation of speciation (inferred from molecular data) in this genus to land availability in New Zealand. Chapter IV looks into the intrageneric relationships, biogeography and population structure of the woodpigeon *Hemiphaga* in the New Zealand region (published in *Journal of Biogeography*). The subsequent chapter (V) focuses on the acridid grasshopper genus *Phaulacridium* that is widespread throughout New Zealand including the Chatham Islands and Australia, in order to assess the influence of spatial scale versus other factors in the development of biogeographic patterns. The last chapter (VI) comprises sequence data from several invertebrate taxa. In this chapter I address the question of how congruent the biogeographic patterns of different taxa are and consider how this informs our understanding of biogeographic assemblages and history of New Zealand's fauna and of biogeography generally. The analysis includes a widespread and well sampled genus of cave weta (*Talitropsis*) as well as a number of variously abundant insect taxa from mainland New Zealand and the Chatham Islands. Each of the chapters is accompanied by its own reference section. Concluding remarks regarding the findings of the different chapters are discussed in the chapter VII, in context of the phylogeography, biogeographic assemblage and evolutionary history of New Zealand's fauna.

Context of the thesis

In this thesis phylogeographic and population genetic analyses in combination with molecular clock approaches are conducted on a diverse set of native animal taxa to gather evidence for patterns of the evolutionary history of the biota of New Zealand. It seeks to elucidate the mechanisms that have yielded the extant distribution and thus the evolutionary processes that have shaped the phylogeographic relationships of species within New Zealand. Phylogeography utilizes spatial distributions of genealogical lineages to infer the influence of historical processes on the evolution of taxa. These methods have the potential to reveal biotic refugia during Pleistocene glacial cycles (and other biodiversity foci) and therefore can be useful in understanding how different lineages responded to changes in climate and habitat availability in the past. Similarly the influence of topographic heterogeneity and land availability can be explored in light of improving information about New Zealand's geological past. What emerges is that there is no common phylogeographic pattern evident among much of the biota of New Zealand indicating that lineages responded differently to biotic and abiotic factors in the past, such as climate cycling, habitat availability, and competition from new taxa. Colonization, speciation and extinction have all played a major role in the assemblage of the biota of New Zealand, and each might be influenced by taxon specific ecological traits and stochasticism.

One of the most intriguing and persistent questions arising is how important vicariance and dispersal were in shaping the extant biota of New Zealand, as they are seen as essential factors in our understanding of the existing biogeographic patterns and evolutionary history of taxa and the biotic assemblage we see today. New Zealand is basically a group of islands isolated from any other land area by more than 1,500 km of ocean. The rocks of New Zealand have been separate from those of Australia and other regions since Zealandia (Trewick *et al.*, 2007) broke from Gondwanaland (ca. 80 Ma), and this has often been taken to imply habitable land has been continuously available since that time. Moreover, it has often been assumed that New Zealand's biota evolved primarily from taxa isolated since ~80 Ma, although the evidence for this has never been more than circumstantial. New Zealand harbours a distinctive biota that includes so-called 'relicts' such as tuatara (*Sphenodon*), leiopelmatid frogs (*Leiopelma*), southern beech (*Nothofagus*) and kauri (*Agathis australis*) (Cooper & Millener, 1993) and their presence has strongly influenced interpretation of New Zealand biogeography. However, it has become evident that diversification and, in many cases, origin of New Zealand taxa postdate the break-up of Gondwanaland (Pole, 1994; McGlone, 2005). This is especially visible through molecular studies of the biota, indicating that diversification in most lineages, and likely arrival of many, has happened relatively recent (Goldberg *et al.*, 2008; Wallis & Trewick, 2009).

This research has substantial significance in the framework of evolutionary theory; rates and modes of speciation, biodiversity and phylogeography, biogeography, efficiency of dispersal and dating of radiation. It also relates to the questions of the origin, age and maintenance of the biota of New Zealand and the interpretation of processes of speciation in general.

Contribution of others to this thesis

This thesis is based on my own work, except where I state the contributions of others. I was responsible for the execution of the different projects and performed the experiments and analyses. Additionally, I carried out fieldwork in New Zealand and on the Chatham Islands and collected many of the invertebrate samples for this project.

So far two of the chapters are published as co-authored papers in peer-reviewed journals, the other chapters are in preparation for submission or in review. Here I would like to mention the contribution of the co-authors:

- In general my supervisors Steven Trewick and Mary Morgan-Richards have been, and will be, co-authors based on their general involvement in these research projects, specifically with discussions and comments on project design, guidance and help during drafting of manuscripts.
- Adrian Paterson is a co-author for chapter II because he was a collaborator in the Chatham Islands project, supplied specimens throughout the study and helped outlining drafts of the paper.
- In chapter IIIa Michael Knapp is a co-author as he introduced me to various sorts of molecular dating methods and gave helpful comments on early drafts.
- Ralph Powlesland is a co-author for chapter IV as he located most of the pigeon samples used in this study and provided helpful ecological insight into the study organism.

II

Evolution of New Zealand's terrestrial fauna: a review of molecular evidence *

ABSTRACT

New Zealand biogeography has been dominated by the knowledge that its geophysical history is continental in nature. The continental crust (Zealandia) from which New Zealand is formed broke from Gondwanaland at about 80 Ma, and there has existed a pervading view that the native biota is primarily a product of this long isolation. However, molecular studies of terrestrial animals and plants in New Zealand indicate that many taxa arrived since isolation of the land, and that diversification in most groups is relatively recent. This is consistent with evidence for species turnover from the fossil record, taxonomic affinity, tectonic evidence and observations of biological composition and interactions. Extinction, colonisation and speciation have yielded a biota in New Zealand that is in most respects more like that of an oceanic archipelago than a continent.

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II.1 INTRODUCTION

New Zealand is a group of islands (270534 km²) isolated by more than 1500 km of ocean from any other significant land area but is continental in stratigraphic composition (see Box 1). Unlike most island systems in the Pacific, understanding the evolution of the New Zealand biota is significantly influenced by continental biogeography (Cowie & Holland, 2006) and has specifically and popularly been described as "Moa's ark" (Bellamy *et al.*, 1990); essentially captive and evolved in isolation for up to 80 million years. However, New Zealand has also been described as an endemism hot spot (Daugherty *et al.*, 1993) with a biota comparable to oceanic islands such as Hawaii and the Galapagos (Gibbs, 2006). It harbours a distinctive biota including 'relict' animal taxa such as tuatara (*Sphenodon*), leiopelmatid frogs, and a Gondwanan element including weta (Orthoptera), peripatus (Onychophora), southern beech (*Nothofagus*) and kauri (*Agathis australis*) (Cooper & Millener, 1993), but it is also home to many recent colonists (Falla, 1953; Fleming, 1979). Though frequently referred to as a Gondwanan element the relevance of this term has been increasingly questioned (e.g. McGlone, 2005). The term "Gondwanan" can be misleading when applied to indicate historical process rather than simply distribution pattern. In the case of the New Zealand biota three alternative meanings can be identified. First, the most restrictive meaning implies that a taxon is Gondwanan if its lineage has been continuously present in New Zealand since rifting from the rest of Gondwanaland. Second, a taxon might also be considered Gondwanan if it is descended from a lineage that was present in Gondwanaland prior to break-up but arrived in New Zealand since Zealandia (see Box 1 and figure 2.1) rifted away from Gondwanaland and sank. Third, Gondwanan might describe a particular type of distribution of lineages in the southern hemisphere with no expectation of a common historical process to explain different instances of this pattern. For example, penguins are found in New Zealand, Australia, southern Africa, South America and Antarctica and certainly demonstrate a Gondwanan distribution (third meaning). Penguins have a fossil history to at least 62 million years ago (Ma) (Slack *et al.*, 2006) and molecular analyses suggest a much earlier origin in the Gondwana landmass (Baker *et al.*, 2006), implying that they might occupy the New Zealand region by virtue of having been in Gondwanaland when Zealandia

Box 1: New Zealand continental origin
The Tasman Ocean which today separates New Zealand from the nearest continent (Australia) was formed 83 – 62 Ma. A section of continental crust moved away from Gondwanaland and this fragment that rifted away is referred to as Zealandia (Campbell & Hutching, 2007; Trewick *et al.,* 2007; Neall & Trewick, 2008). In geological terms, 'continental' means composed of continental rather than oceanic crust, but for continental crust to be emergent as land it has to be thick enough to stand above sea level. The Zealandia fragment was much larger than modern New Zealand, about the size of India, and included the Campbell Plateau, Challenger Plateau, Lord Howe Rise, Norfolk Ridge, Chatham Rise and New Caledonia. As it rifted, Zealandia was stretched and thinned, effectively losing buoyancy, and sinking some 2-3,000 metres. Today, about 93% of Zealandia is beneath the sea, and in the Oligocene the New Zealand region may have also been entirely submerged (Landis *et al*., 2008). New Zealand exists above water today because of a plate boundary collision. It has been vigorous and sustained since its abrupt initiation in latest Oligocene time (Sutherland, 1999; Cande & Stock, 2004). This activity is prominently expressed along the alpine fault, where tectonics have generated 480 km of lateral motion and, since the Pliocene, 20 km of uplift resulting in the formation of the Southern Alps (Kamp *et al.*, 1989; Kamp, 1992; Whitehouse & Pearce, 1992). Today, the continental crust of Zealandia, including New Zealand, is in geological terms thin (20-25 km) and New Zealand may be treated as a comparitively old oceanic island group (Trewick *et al.*, 2007). The geological evidence for the survival of any ancient terrestrial parts of Zealandia is unexpectedly weak, due in part to the destructive impact of later tectonics, questioning the assumptions on the ancient origin of New Zealand's biota (Landis *et al*., 2008).

rifted away (first meaning). But, modern extant penguins are monophyletic and fossil and molecular data suggest they evolved in the early Oligocene (Baker *et al.*, 2006) and extant penguin genera of *Eudyptes*, *Eudyptula* and *Megadyptes* may have colonised New Zealand from other former Gondwanan landmasses since that time (second meaning). Indeed, *Eudyptula*, the blue penguin, is found in Australia and New Zealand with evidence of recent contact between these populations (Banks *et al.*, 2002). Thus the modern distribution of penguins could be described as Gondwanan in all three senses. Distributional data are clearly not indicative of historical processes alone and therefore the presence of lineages in New Zealand cannot in itself be taken as evidence of a dominant role of continental drift in the origination of the biota. Unfortunately, it is just this supposition that is widely made. The addition of timing of divergence is necessary to choose between the three meanings of Gondwanan.

Figure 2.1: The continent of Zealandia (modified from Stagpoole, 2002).

In recent years it has become increasingly apparent that diversification, and in many cases, origin of New Zealand taxa substantially postdate the break-up of Gondwanaland (Pole, 1994; McGlone, 2005; McDowall, 2008). The major impetus for the resurgence of dispersal as an accepted contributing factor in the formation of the biota comes from molecular studies. Because of the interesting biotic assemblage present in New Zealand, the question of whether New Zealand should be treated as an island or a micro-continent (Daugherty *et al.*, 1993) has led to many studies on speciation and colonization which focus on New Zealand's biota (Gillespie & Roderick, 2002) and its relationship to other close landmasses (i.e. Australia, New Caledonia) (see Sanmartin & Ronquist, 2004; Cook & Crisp, 2005).

(a) *Illogical juxtaposition*

So attractive has the notion of an ancient ("Gondwanan") insular biota been, that it is frequently, though illogically, juxtaposed with observations of the composition of the fauna and flora, that have in the past been recognised as consistent with island biota subjected to dispersal (Wallace, 1876; Falla, 1953; Darlington, 1957, 1965; Fleming, 1962*a*, 1963*a*; Caughley, 1964; Gaskin, 1970, 1975; Raven, 1973; Cracraft, 1974, 1975).

Several influential though not necessarily meaningful observations have been made about New Zealand and its biota: 1) it is isolated from other landmasses, 2) the biota is unique, 3) it has high endemicity, 4) it includes behaviourally or morphologically strange and distinctive taxa, and 5) the composition of New Zealand biota is disharmonic (Gibbs, 2006; McDowall, 2008).

"…its whole biota is anomalous, depauperate, and rather different from that of Australia." (Keast, 1971, p.359).

"Although New Zealand enjoys a rich, unique biodiversity, it can equally well be described as 'naturally depauperate', meaning that many of the types of animals and plants that one might expect to find here are absent.' (Gibbs, 2006, p.20)

Since the rise of vicariance biogeography and acceptance of continental drift (e.g. Skipworth, 1974; and see Waters & Craw, 2006 and references therein), observations of this type have been interpreted as evidence of an old island with a biota that has been isolated for a very long time (i.e. 80 million years). However, they are actually what might be expected from the fauna of a young oceanic island and/or a high level of extinction. Isolation ensures that only a subset of nearby continental faunas will be represented on the islands and the lack of great age of the island would account for the low diversity levels. Very commonly, the New Zealand biota is described as "unique" and unlike anything elsewhere. Diamond (1997) described the biology as "the nearest approach to life on another planet". In fact, the nature of the biota is inconsistent with the process to which it is frequently ascribed because,

(1) physical isolation does not equate to biological isolation,

(2) all biotas are unique,
"Islands resemble one another in that each is unique" (Quammen, 1996)

(3) species endemicity is usually high on oceanic islands,
"New Zealand ranks alongside island groups like Hawaii and the Galapagos Islands for its levels of endemism." (Gibbs, 2006, p.12)

(4) distinctive taxa are common products of evolution on islands, and

(5) disharmonic biotas are best and usually explained as resulting from stochastic colonisation, and extinction (Carlquist, 1965).
"Trans-oceanic dispersal by air and water from neighbouring continental areas and islands was thought to have played quite an important role at all times in New Zealand's history in assembly of the disharmonic fauna and flora of the Archipelago" (Gaskin, 1975, p.87).

If New Zealand was isolated for 80 million years we would expect it to support the descendants of a Zealandian biota with high diversity, complex coevolutionary associations, endemicity at deeper taxonomic/phylogenetic levels, and a more complete faunal composition.

(b) ***Ancient lineages and living fossils***

Several "ancient" lineages have been identified within the New Zealand biota. For example, the tuatara (*Sphenodon*) is a relict of the sister group to the squamate reptiles and has an independent history of over 250 million years (Hugall *et al.*, 2007). Other ancient endemic lineages are the leiopelmatid frogs (Estes & Reig, 1973; Roelants & Bossuyt, 2005) and acanthisittid wrens (Ericson *et al.*, 2002). The presence of these lineages in New Zealand, but not elsewhere, is tantalising evidence that Gondwanan lineages have persisted in the New Zealand region since the break-up of Gondwanaland (Gibbs, 2006). However, on their own, ancient lineages tell us very little about their longevity in the region as endemism on islands can arise in several ways (Emerson & Kolm, 2005). Lineages now endemic to New Zealand might have been present in Australia for instance but have gone extinct there or they may have speciated within either Australia or New Zealand. Neither of these scenarios is age dependent in that they could have occurred in lineages present since the break-up of Gondwanaland or after more recent colonisation of New Zealand. For example, the tuatara lineage may have been present since the break-up in New Zealand but subsequently gone extinct in Australia (and elsewhere) or it may have colonised New Zealand any time over the last 80 million years and subsequently gone extinct in Australia. 'Ancient lineages' tend to be recognised as such by the absence of close relatives but this presents a problem when inferring their history. The tuatara and its closest living relatives have a common ancestor over 100 million years before New Zealand broke away from Gondwanaland (Hugall *et al.*, 2007) but this clearly does not inform us about their biogeographical history (Crisp & Cook, 2005). The absence of fossils, or lack of reliably time-constrained or taxonomically precise fossils also limits inferences.

A New Zealand lineage might be found, after careful calibration of genetic divergences, to have a common ancestor with a nearest relative in Australia dating to about 80 Ma. This would imply the lineage has been present in New Zealand since break-up, but a more closely-related lineage in Australia might have been extinguished. If so, the evidence for a post break-up dispersal history would have been lost. For instance morphological (Parrish *et al.*, 1998) and DNA (Stöckler *et al.*, 2002; Knapp *et al.*, 2007) studies are consistent with the conifer genus *Agathis* (Araucariaceae), having been continuously present in New Zealand since Cretaceous time, and living New Zealand kauri, *Agathis australis,* appears to be sister to other living *Agathis* in New Guinea, Australia and New Caledonia (Landis *et al.*, 2008). This pattern is consistent with an ancient vicariant origin in New Zealand, and is probably the best example of any ancient element supported by molecular evidence. However, a single sister species of *Agathis australis* in Australia would falsify that inference; and such a sister lineage might have existed until recently but now be extinct and unavailable for analysis (figure 2.2). In fact the oldest fossils of *Agathis* in New Zealand are Late Oligocene / Early Miocene (Lee *et al.*, 2007) and the oldest

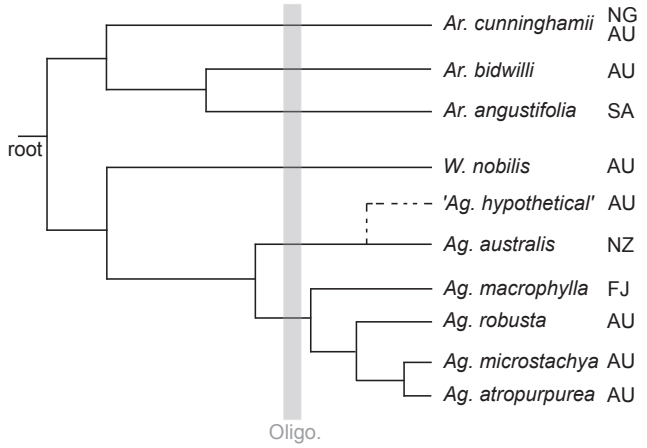

Figure 2.2: Historical inference from phylogenetic trees is sensitive to tree shape and taxon sampling (see Crisp & Cook, 2005). For instance, a single putative undiscovered or recently extinct Australian lineage closely related to the extant New Zealand species *Agathis australis* ('*Ag. hypothetical*', dashed line) would yield a completely different history of the group (tree modified from Knapp *et al.*, 2007) and falsify the inference that *A. australis* is "the sole representative of an early diverged lineage within the genus". (NG= New Guinea; NZ = New Zealand; AU = Australia; SA = South America; FJ = Tropical Australasia; Oligo. = Oligocene).

pollen fossils of *A. australis* date to the Pliocene / Pleistocene and there are no records of similar Cenozoic araucarian fossils younger than Early Miocene (Pole, 2001); evidence that is consistent with both a post-Gondwanan colonisation scenario and a vicariant Gondwanaland history (Waters & Craw, 2006). The issue of whether fossil absences are real or not is, therefore, crucial in interpreting the biogeographical history of extant lineages. So do relict taxa tell us anything about the biogeographical history of New Zealand? Individually, each taxon adds just one datum. An overwhelming number of relict taxa might be compelling. However, there is no research examining how many relictual taxa we might reasonably expect to see after 80 million years for us to assess whether the number that we identify in New Zealand is more or less than this expected number.

New fossil evidence of a Miocene mouse-size mammal from South Island, New Zealand has important implications for the biogeography of New Zealand (Worthy *et al.*, 2006), as the Holocene biota lacks native terrestrial mammals except three bats. This find clearly illustrates the importance of 'missing' fossils. Prior to 2006, the biogeographical history of New Zealand had been presented as one without mammals with many inferences that the flora and fauna had evolved over 80 million years in response to this absence (e.g. Wilson, 2004). Now it is evident that there was a mammalian fauna for at least part of New Zealand's history (Worthy *et al.*, 2006). The subsequent (pre-human) extinction of mammals and other lineages since the Miocene reveals that, whatever the origin of New Zealand biota, it had been subjected to major extinction and replacement events (e.g. Pole, 2001; Lee *et al.*, 2001). This alone demonstrates that to attribute the extant biota primarily to an ancient vicariant event (Gondwanan break-up) is fraught with difficulties.

II.2 Spatial paradigms in New Zealand biogeography

Here we examine how molecular studies of New Zealand taxa have shed light on the origins and development of the biota. For convenience we approach this at six spatial/ecological levels, although some studies inform at more than one. At each level, we identify one or more taxonomic exemplars that illustrate available evidence, focusing our attention on the terrestrial fauna but referring also to additional relevant studies for comparison, including plant taxa and freshwater

taxa where this makes a pertinent contribution. An important feature of molecular studies is their capacity, with appropriate calibration, to provide estimates for the timing of phylogenetic events. However molecular clocks have their limitations. The strict molecular method (using a fixed rate of molecular evolution) is often employed when fossils or geographical calibrations are not available, and researchers generally treat resulting date estimates cautiously. A strict clock does not take into account the variation in rates of molecular evolution that exist among genes, taxonomic groups and across time (Avise, 2004). Relaxed molecular clock methods attempt to accommodate minor rate variation over time and within lineages (Kishino *et al.*, 2001). Here we report inferences made by the original authors and treat conservatively the timing of phylogenetic events indicated by level of DNA sequence divergence.

(a) ***New Zealand and "Gondwana"***

The New Zealand landscape is essentially the product of tectonic activity initiated ~25 Ma. It is far from certain how much land persisted in the region prior to this time as the continental crust (called Zealandia, Box 1) thinned and submerged beneath the sea surface after the separation from Gondwanaland starting about 83 Ma (Campbell & Hutching, 2007; Trewick *et al.*, 2007; Neall & Trewick, 2008). It is clear that extensive land reduction took place (Landis *et al.*, 2006*,* 2008) and this period in New Zealand's natural history is thought to have had a major influence on the subsequent composition of the biota (Cooper & Cooper, 1995).

Despite the fact that several molecular studies now implicate colonisation of New Zealand by a diverse range of animals and plants (e.g. bowerbirds, Christidis *et al.*, 1996; *Sophora*, Hurr *et al.*, 1999; insects, Trewick, 2000*a*; freshwater fish, Waters *et al.*, 2000; parakeets, Boon *et al.*, 2001*a*, 2001*b*; cicadas, Buckley *et al.*, 2002, Arensburger *et al.*, 2004*b*; hebe, alpine buttercups, forget-me-nots, Winkworth *et al.*, 2002; short-tailed bats, Teeling *et al*., 2003; southern beech, Knapp *et al.*, 2005; plants, de Queiroz, 2005; ferns and other plants, Perrie & Brownsey, 2007), there is still a prevailing sense that the most important components of the New Zealand biota are of ancient vicariant origin and that New Zealand's biota is very different from other island biota due to this ancient origin.

(i) Ratites

Among large vertebrates, which are rare in New Zealand, the ratites (Palaeognaths) have long been seen as having a classic "Gondwana" distribution (Craw *et al.*, 1999), with representatives in Australia, New Zealand, Africa, South America, but interestingly not India or New Caledonia. New Zealand is unusual in having two quite distinct groups of ratites (moa and kiwi) although the fossil record for these extends no further than late Pliocene (Worthy & Holdaway, 2002). Despite being extinct, molecular data has been gleaned from numerous Holocene fossil bones of moa. Analysis indicates that moa form a monophyletic group among ratites (Haddrath & Baker, 2001; Cooper *et al.*, 2001; Worthy & Holdaway, 2002) and that much of the morphological diversity at one time was attributed to 64 species (in 20 genera) actually represents sexual dimorphism among eleven species (Bunce *et al.*, 2003; Huynen *et al.*, 2003). Moa are, however,

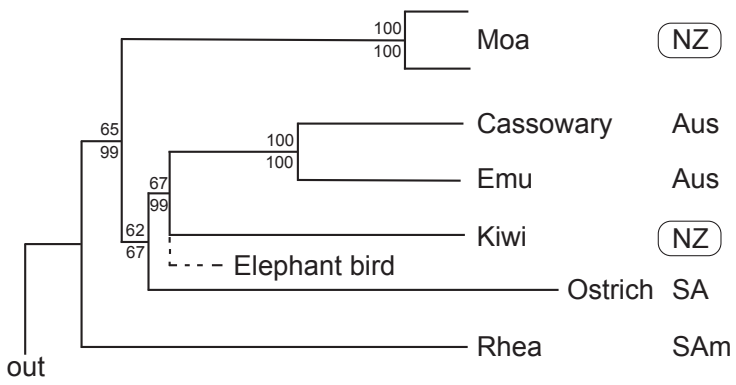

Figure 2.3: Phylogeny of the ratites (redrawn from Cooper *et al.*, 2001) emphasizing that New Zealand taxa (moa and kiwi) are not monophyletic (NZ = New Zealand; Aus = Australia; SA = South Africa; SAm = South America).

not monophyletic with the kiwi, which appear to share a closer common ancestor with the Australian emu leading to the inference that at least some ratites may have migrated to New Zealand after separation of Zealandia (Cooper *et al.*, 2001) (figure 2.3). Currently moa do not appear to have close allies among any extant ratites, and this pattern could be consistent with a vicariant origin. However, the species radiation of both moa (Baker *et al.*, 2005) and kiwi is relatively young (Baker *et al.*, 1995; Burbidge *et al*., 2003). This is despite the assumption that moa ancestors have been in New Zealand since isolation from Australia (Baker *et al.*, 2005).

(ii) Harvestmen

The Pettalidae family of morphologically conserved harvestmen are found in leaf litter and have a classic Gondwanan distribution (Boyer & Giribet, 2007). Despite finding that most groups within this clade form monophyletic continental groups, New Zealand is home to three different lineages represented by the genera *Neopurcellia*, *Rakaia* and *Aoraki*, that are largely structured by South Island mountain uplift (Boyer & Giribet, 2007). Contrary to the inference of a vicariant history to explain this pattern, the levels of molecular divergence among the pettalid lineages are too low to be consistent with an ancient origin, unless there has been a very substantial taxon specific change in mutation rate. Diversity and spatial structuring on South Island mountains that are about 5 million years suggest an arrival of these lineages within the last few million years.

(*iii) Other examples*

In recent years other examples of so-called 'ancient Gondwanan lineages' have been shown to have dispersed and speciated after the break-up, including animal groups, such as galaxiid fish (Waters *et al.*, 2000) and wattlebirds (Shepherd & Lambert, 2007), as well as plants, such as southern beech (Knapp *et al.*, 2005). The implication of these findings is that the Gondwanan element of the biota of New Zealand is primarily of type 2 (see above, i.e. of southern distribution but not vicariant origin).

(b) ***New Zealand and Australia***

Linkages between the fauna of New Zealand and Australia have long been recognised (Falla, 1953; Fleming, 1962*a*, 1962*b*, 1979) and key taxa include weta (Orthoptera), peripatus

(Onychophora), plus iconic plant taxa such as southern beech (*Nothofagus*) and kauri (*Agathis australis*). The extinct giant New Zealand eagle (*Harpagornis*) that once hunted moa, was closely related to one of the smallest extant Australian eagles (*Hieraaetus*). Genetic distances measured from DNA of subfossil bones indicate morphogenesis of this eagle lineage after colonisation of New Zealand during the Pleistocene, approximately 0.7–1.8 Ma (Bunce *et al.*, 2005).

(i) Hepialid moths

The moth family Hepialidae is found in Australia, New Guinea and New Zealand. Within New Zealand there has been a radiation of this forest group out into grasslands, particularly in the sub-alpine zone and recently into exotic pasture where it has become an important pest species. Brown *et al.* (1999) found that at least two hepialid lineages had dispersed from Australia to New Zealand successfully over the last 4-5 million years coinciding with uplift along the alpine fault.

(ii) Spiders

Latrodectus widow spiders have a global distribution and New Zealand and Australia have the endemic katipo (*L. katipo*) and red-back (*L. hasselti*) species respectively. Griffith *et al.* (2005) found genetic divergence between katipo and red-backs was equivalent to two very closely related species and that katipo are relatively recent arrivals into New Zealand. Likewise, the diverse Lycosid wolf spiders of New Zealand share a close relationship to Australian relatives and show a New Zealand species radiation (~20 species) no older than 5 million years (Vink & Paterson, 2003).

(*iii*) *Other examples*

Other examples of the strong and often recent connection of the New Zealand biota to Australia are many bird species (Falla, 1953). The silvereye (*Zosterops lateralis*) was self-introduced from Australia in the mid 1900s and has since colonized the surrounding islands (Clegg *et al.*, 2002). Interestingly, range expansion of silvereye within New Zealand has resulted in gradual reduction in allelic diversity, whereas the initial oversea colonisation brought high genetic diversity consistent with the arrival of a flock rather than few individuals (Clegg *et al*., 2002). Slightly earlier arrivals include the Petroicidae (Australasian robins), which appear to have colonized New Zealand from Australia in two separate events in relatively recent times (Miller, 2003). Intriguingly, even the iconic Onychophora (peripatus or velvet worms) show an unexpected phylogenetic pattern. Although based on rather few data, the New Zealand onychophoran fauna appears to be more closely allied to that of Tasmania than either are to Australian species, despite the closer proximity and recent connection of Australia and Tasmania (Gleeson *et al*., 1998).

(c) ***New Zealand in the Pacific***

A range of New Zealand animal taxa including snails, land birds, seabirds as well as plants such as *Metrosideros* have their clostest living relatives on islands of the Pacific (Fleming, 1979). Molecular analysis of *Metrosideros* indicates dispersal and speciation since the Pliocene (Wright *et al.*, 2000; Percy *et al.*, 2008) and complex patterns indicative of multiple dispersal events have been found in several other plant groups in the region (e.g. Bartish *et al.*, 2005; Harbaugh & Baldwin, 2007).

(i) Landsnails

Pulmonate land snails of the genus *Placostylus* are found only in the Western Pacific, in northern New Zealand and on islands between New Zealand and Melanesia (Solomon Islands, Fiji, New Caledonia, Vanuatu, Papua New Guinea and Lord Howe) (Suter, 1916; Ponder *et al*., 2003). Despite the fact that most of these islands emerged from beneath the ocean, this distribution has been considered by some researchers to be consistent with an ancient Gondwanan origin (Stanisic, 1981). The large size of these taxa and their intolerance of sea water has generally led to the assumption that *Placostylus* are unlikely to disperse across the ocean. However, *Placostylus* arrived on Lord Howe after its formation (<7 Ma, McDougall *et al.*, 1981), and New Caledonian *Placostylus* have dispersed to the nearby Loyalty Islands within the last 2 million years. Comparison of sequence data from New Caledonian *Placostylus* and representatives of the genus from New Zealand and Lord Howe Island, indicates that the New Caledonian radiation may have originated by dispersal from these southern locations (Ponder *et al.*, 2003; Trewick *et al.*, 2008*a*).

(ii) Cormorants and shags

The Phalacrocoracidae (cormorants and shags) are a prominent component of New Zealand's seabird fauna. There are two main lineages of shags in New Zealand, the king shag species complex and the cliff shags. Both lineages have close links into the Pacific and even further afield. The king shags, including *Phalacrocorax chalconotus* from Stewart Island and several sub-Antarctic species are, on the basis of DNA sequence divergence, very closely related to *P. bougainvillii* from southern South America (Kennedy *et al.*, 2000). The New Zealand spotted shag, *Stictocarbo punctatus*, is also closely related to other species from Australia, Japan, Africa and Europe (Kennedy *et al.*, 2000).

(iii) Other examples

Further examples for Pacific connections, post-dating Gondwanaland, are found in many animal lineages. Among cicada there is a strong and recent affinity of the New Zealand cicada fauna to taxa in other parts of the Pacific region including Norfolk Island, Chatham Islands, Australian and New Caledonian taxa (Arensburger *et al*., 2004*a*, 2004*b*). The freshwater shrimp (*Paratya*) has colonised islands from Japan to New Zealand since 19 Ma (Page *et al*., 2005). Lizards including skinks (Smith *et al.*, 2007), and geckos (Chambers *et al.*, 2001), and birds such as

parakeets (Chambers *et al.*, 2001; Boon *et al.*, 2001*a*, 2001*b*) and robins (Miller, 2003) show similar Pacific connection, especially to New Caledonia. The wandering albatross complex that includes species in New Zealand and islands in the region shows extremely low levels of genetic diversity (Burg & Croxall, 2004). Though many are extinct, the Rallidae (*Gallirallus* and *Porphyrio*) are represented in New Zealand and Chatham Islands (as on most oceanic islands, Steadman, 2006) by numerous flightless endemics, each having evolved following colonisation (Trewick, 1997*a*, 1997*b*). New Zealand weta (crickets) are allied to biotas of Australia and New Caledonia (see Pratt *et al*., 2008), and the New Zealand stick insects form a monophyletic group with relatives in the same region (Trewick *et al*., 2008*b*).

(d) ***New Zealand - Chatham Islands***

The Chathams Islands are a small archipelago c. 850 km to the east of New Zealand (comprising 5 islands and several small islets, 970 km^2 land area in total) (figure 2.4a). Like New Zealand, it is formed from continental crust supplemented locally by volcanic sediments, and some have assumed it to be an ancient Gondwanan land surface. Craw (1988) presented a panbiogeographic thesis that found an explanation for the assemblage of the biota in the perceived composite nature of geological terrains (northern and southern elements).

Geological evidence indicates that the Chatham archipelago emerged from the sea in the last 4 million years (Campbell, 1998; Campbell *et al.*, 2006). Genetic evidence from invertebrate taxa so far studied are consistent with this inference (Trewick, 2000*a*; Chinn & Gemmel, 2004; Arensburger *et al.*, 2004*b*; Hill *et al.*, 2005; Trewick *et al.*, 2005; Paterson *et al.*, 2006). A relatively young biota is also evident from studies of plants (Wagstaff & Garnock-Jones, 1998) and vertebrates such as rails (Trewick, 1997*b*), parakeets (Boon *et al.*, 2001*a*, 2001*b*), pigeons (Millener & Powlesland, 2001) and robins (Miller, 2003). Endemism is almost entirely confined to species level or below. Naturally the biota of the Chathams is "unique", a particular assemblage of lineages including a wide variety of organismal groups including large flightless beetles and crickets, freshwater fish and flightless birds. Despite a recent origin the level of speciation and genetic structure on the islands can be impressive, as can be seen in the cave weta genus *Talitropsis* (Raphidophoridae) (figure 2.4b). It comprises three species, two of which are endemic to the Chatham Islands (*T. megatibia* & *T. crassicruris*) and one that is widespread throughout both islands of New Zealand (*T. sedilloti*). Despite the low genetic divergences within the islands and between New Zealand and the Chatham Islands, the species on the Chathams still show a high level of haplotype diversity and cladogenesis consistent with the two endemic species (Trewick, 1999, 2000*a*; Goldberg, unpubl.). Other invertebrates like the earwig species *Anisolabis littorea* show no morphological and minimal genetic differentiation from New Zealand populations.

The Chatham Islands provide an important lesson in how rapidly a "unique" biota can evolve from dispersal in the New Zealand region. Clearly, evidence from species endemicity is no justification for thinking that much of the native fauna of New Zealand could not have originated within the last 25 million years after the peak of marine inundation, to occupy free niche space.

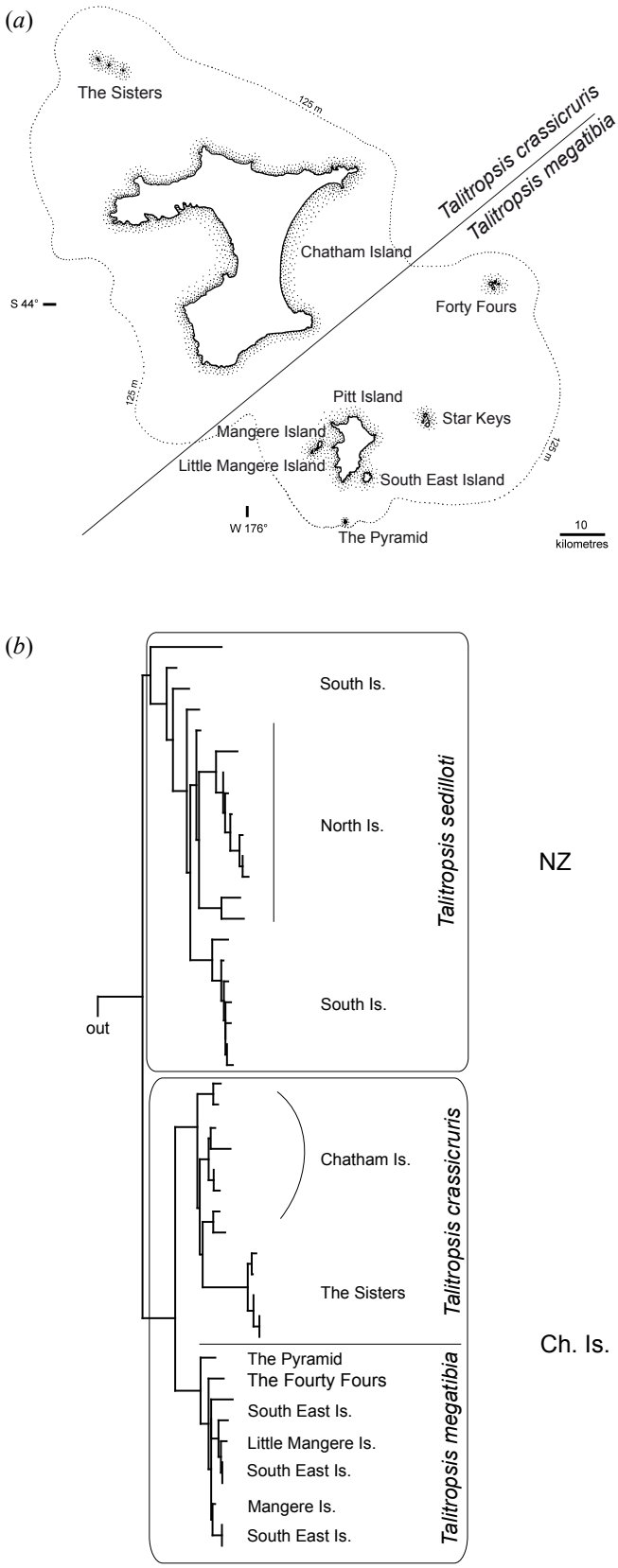

Figure 2.4a: Map of the Chatham Islands with the distribution of two species of the cave weta genus *Talitropsis* on the archipelago. The broken line indicates the presumed land area above sea level during the ice age. (b) The phylogeny of *Talitropsis* in New Zealand and the Chatham Islands highlighting the haplotype diversity on the geologically young Chatham Islands (max. 4 million years, Campbell *et al.*, 2006). Low levels of variation in mitochondrial COI data between New Zealand and Chatham species and within the Chatham Islands (Goldberg, unpublished) corroborates earlier results for Talitropsis (Trewick, 2000*a*) and emphasizes that speciation can happen relatively fast even in a geographically small landscape. (NZ = New Zealand; Ch. Is. = Chatham Islands).

(e) ***Alpine New Zealand***

The mountains of New Zealand are very young in comparison to the time since isolation of Zealandia. The alpine ranges of the South Island developed by extreme crustal uplift and orogenesis since the Pliocene (Kamp, 1992) resulting in a substantial area of mountainous and ecologically diverse habitat. These South Island ranges in particular have a diverse and extensive alpine biota (e.g. Fleming, 1963*b*; Gibbs, 2006) including insect taxa that are freeze tolerant (Sinclair *et al*., 1999). Their presence poses the question of the origin of this biota. Fleming (1963*b*) extended two alternatives: (a) colonisation of New Zealand by cold adapted taxa from elsewhere (e.g. Australia (Raven, 1973), Antarctica (Hooker, 1860), or northern boreal habitats), and (b) radiation and adaptation in New Zealand during the Pleistocene glacial epoch. A third alternative can be added: evolution in New Zealand in response to the development of an alpine zone on mountain ranges that emerged during the Pliocene. In the North Island the ranges are less extensive and even younger (< 1 million years) than those in South Island. The Northern alpine biota is less diverse and primarily a subset of its southern counterpart. It is becoming increasingly evident from molecular studies that the alpine biota has evolved in response to mountain habitat development and cyclic expansion of the alpine zones during the Pleistocene.

(i) Scree weta

The alpine scree weta (*Deinacrida connectens*) is one of the most striking alpine insects of New Zealand. It is a large, flightless species belonging to the Anostostomatidae (Orthoptera), which has a classic "Gondwanan" distribution. *Deinacrida connectens* lives only above the treeline in alpine scree slopes of the Southern Alps (South Island). Mitochondrial COI sequence data revealed comparatively high genetic distances among populations of *D. connectens* (Trewick *et al.*, 2000) although nuclear markers indicate this does not reflect the presence of cryptic species (Morgan-Richards & Gibbs, 1996). Population structure appears instead to represent restricted gene flow among mountain ranges and may date back to Pliocene uplift of the Southern Alps (Trewick *et al.*, 2000; Trewick, 2001*a*, 2001*b*).

(ii) Cicadas

The endemic cicada genus *Maoricicada* is an alpine specialist with the majority of described species occupying alpine or subalpine habitats. Comparison of sequence data and relaxed molecular clock dating reveals that speciation of alpine *Maoricicada* falls within the timeframe of the Pliocene uplift of the Southern Alps (~5 Ma) (Buckley & Simon, 2007). Additionally the phylogeographic structure within the alpine species *M. campbelli* in the Southern Alps and North Island mountains dates to the Pleistocene (Buckley *et al.*, 2001) (figure 2.5).

(iii) Other examples

Other examples of terrestrial species radiations (adaptive or otherwise) in New Zealand are associated with alpine habitats, mostly in South Island. These include invertebrates (tree

and giant weta, Morgan-Richards & Gibbs, 2001 and Trewick & Morgan-Richards, 2005; *Brachaspis* and *Sigaus* grasshoppers, Trewick, 2001*a*, 2007; *Celatoblatta* cockroaches, Trewick & Wallis, 2001 and Chinn & Gemmel, 2004; *Powelliphanta* snails, Trewick *et al.*, 2008*c*), vertebrates (skinks, Hickson *et al.*, 2000; moa, Baker *et al.*, 2005), and many alpine plants (*Hebe*, Wagstaff & Garnock-Jones, 1998; *Ranunculus* buttercups, Lockhart *et al.*, 2001; *Pachycladon*, Heenan & Mitchell, 2003 and Winkworth *et al.*, 2005; *Ourisia*, Meudt & Simpson, 2006).

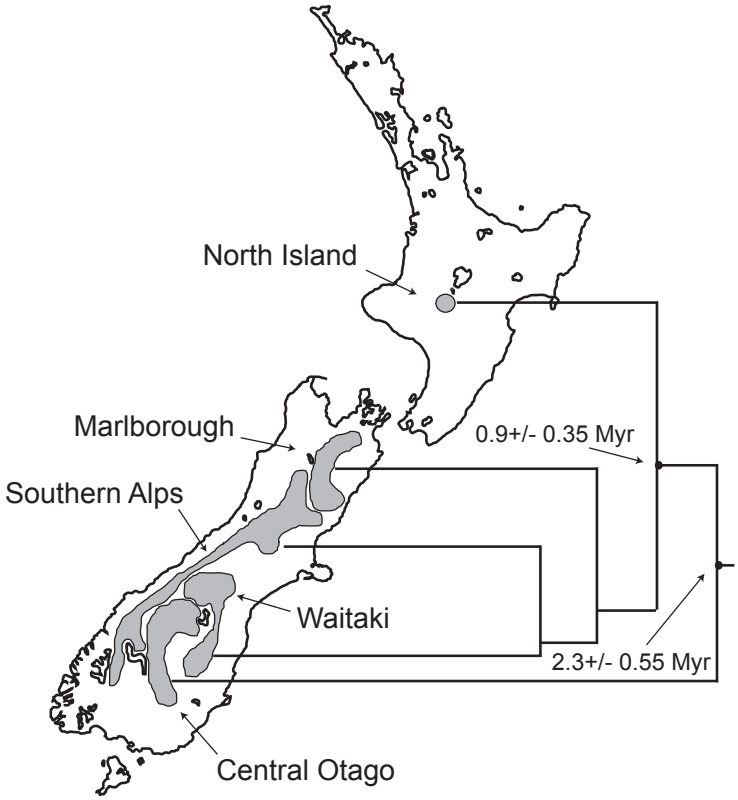

Figure 2.5: Showing the phylogeographic structure of the alpine cicada *Maoricicada campbelli* based on mitochondrial sequences. Timing of lineage formation in the mountain ranges of the Southern Alps correlates with Pleistocene/Pliocene orogenics (modified from Buckley *et al.*, 2001).

(f) *Lowland New Zealand*

Pre-human New Zealand in the Holocene was dominated by mixed temperate to subtropical forests (Trewick & Morgan-Richards, 2009) most of which have been cleared since human settlement. Palaeoecological reconstructions reveal that during Pleistocene glacials forest was reduced and largely restricted to northern New Zealand (McGlone *et al.*, 2001). The extent, composition and number of other forest remnants during the glacials are unclear but it is likely that there were some, most probably in the northwest of South Island of New Zealand (Alloway *et al*., 2007). During glacials, lowland New Zealand was dominated by grass and scrub, so it is reasonable to expect that populations of forest animals experienced severe reduction during those episodes (Trewick & Wallis, 2001). Prior to the Pleistocene, the configuration of islands in the New Zealand archipelago was quite different from that of today, with, in particular, numerous small islands in the north during the Pliocene (Fleming, 1979; Isaac *et al.*, 1994; Balance & Williams, 1992). A prominent biogeographic feature in South Island, is the north-south disjunction of many species distributions and this pattern, referred to as the "beech gap" has been attributed to vicariant separation by movement along the Alpine fault that extends through the island (Wallis & Trewick, 2001). However, molecular evidence from a wide range of invertebrate taxa reveal phylogenetic patterns and levels of genetic diversity that are inconsistent with this inference (Trewick & Wallis, 2001). Disjunctions are better explained by local extinction and range expansion during and since the Pleistocene (e.g. Trewick & Wallis, 2001; Leschen *et al.*, 2008; Hill *et al.*, 2009).

(i) Kauri snails

Charles Fleming (1979) proposed that taxa in Northern New Zealand may have subdivided and speciated in response to presence of Pliocene islands that subsequently united to form Northland, New Zealand. Carnivorous snails of the Rhytididae have an intriguing distribution that encompasses Gondwanan landmasses as well as Pacific Island groups. The subfamily Paryphantinae contains four genera, including the kauri snails, *Paryphanta*, and is almost entirely limited to Northland and some off-shore islands in this region and is among taxa that might have experience population subdvision during the Pleistocene (Fleming, 1979). Spencer *et al.* (2006) found that speciation probably did happen in the Pliocene but they failed to find spatial patterns consistent with Pliocene vicariance events. A similar complex pattern developed since the Pliocene exists among *Oligosoma* skinks in the region (Hare *et al*., 2008).

(ii) Tree weta

The New Zealand tree weta *Hemideina thoracica* is an arboreal herbivore. It is flightless like all other Anostostomatidae (Orthoptera) species in New Zealand, and it is confined to North Island, New Zealand. Mitochondrial DNA sequences revealed higher genetic diversity in northern populations of *H. thoracica* than in the south of the island (Morgan-Richards *et al.*, 2001) and it indicated that genetic diversity, genetic distances, spatial distribution of mitochondrial lineages and chromosome races are consistent with simultaneous formation of at least five isolated populations on Pliocene islands in northern New Zealand (figure 2.6).

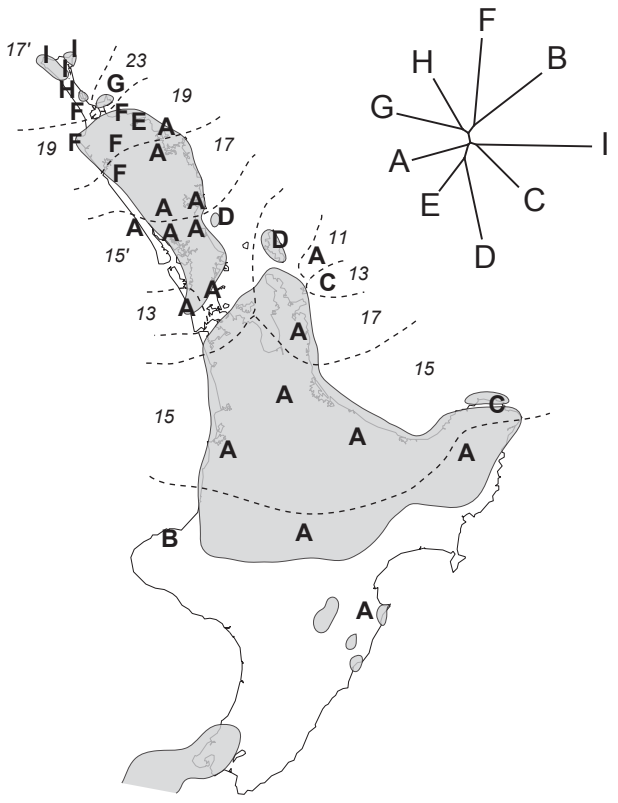

Figure 2.6: North Island, New Zealand with the location of land during the Pliocene (grey area). The distribution (ranges indicated by dashed lines) of eight chromosome races of the tree weta *Hemideina thoracica.* Values 11, 13, 15 etc. are male diploid chromosome numbers. The distribution of and nine well-differentiated mitochondrial (COI) lineages (A–I) are also indicated and support differentiation of chromosome races 5.3-1.8 Ma during the Pliocene (from Morgan-Richards *et al.*, 2001).

(iii) Other examples

Several New Zealand insect groups have distributions that extend from North Island into northern South Island, which are indicative of recent range expansion (e.g. *Clitarchus* stick insects, Trewick *et al.*, 2005; tree weta, Trewick & Morgan-Richards, 2005). Such range shifts probably reflect shifting habitat availability following the last Pleistocene glaciation (i.e. warming climate and southwards expansion of forests), although some appear to be earlier than late Pleistocene

(freshwater crayfish, Apte *et al*., 2007). Flying forest birds, (e.g. parakeets, Boon *et al.*, 2001*a*; Australasian robins, Miller, 2003; kokako, Murphy *et al*., 2006; wood pigeons, Goldberg *et al.,* 2011) and the endemic short-tailed bat (Lloyd, 2003) also have low levels of genetic variation among populations suggesting they too suffered intense bottlenecking during the mid to late Pleistocene. Cicadas of the genus *Kikihia* are found throughout New Zealand in many lowland habitats and several species have moved into the subalpine region but none are alpine. Relaxed molecular clock dating indicates that the major radiation in *Kikihia* species happened at about 4 Ma (Arensburger *et al.*, 2004*b*; Marshall *et al.*, 2008). However, some invertebrates, including peripatus (Trewick, 2000*b*) and giant spring-tails (*Holacanthella*) (Stevens *et al.*, 2007), are more species rich and have more complex and often sympatric distributions over the same landscape, or have high within-group genetic diversity (mite harvestmen, Boyer *et al*., 2007). This suggests an older history with local survival of populations through climate cycles, and this pattern may reflect the distinctive reproductive systems and population structure of these invertebrates that have narrow habitat requirements. The genetic diversity of the flightless brown kiwi (*Apteryx*) is more like these latter invertebrates, with five main spatially partitioned lineages indicating local persistence through climate and thus vegetation cycles of the Pleistocene (Baker *et al.*, 1995; Burbidge *et al*., 2003).

II.3 DISCUSSION

Biogeographic interpretations based only on observations of animal distributions are often misleadling, but the inclusion of molecular evidence has added a vital temporal dimension to biogeographic analysis and evolutionary study in general. The temporal dimension enables the likelihood of alternative explanations for a given typology of species-area relationships to be assessed. For instance, the alternative inferences of "Gondwanan" distributions discussed earlier could be distinguished in this way. A long-standing debate has focused on the dichotomy of vicariance and dispersal influences on New Zealand's biology, but a much more productive approach is to explore data on a case by case basis and to recognise that some, and perhaps many, instances yield equivocal results (McGlone, 2005). In the search for unifying concepts biogeographers have tended to oversimplify the history of New Zealand, which is more complicated and less well understood than that of typical continents and typical oceanic islands. This complexity, with a combination of geophysical features characteristic of both continents and oceanic islands (Daugherty *et al.*, 1993), is very probably a major source of the "unique" qualities of New Zealand's biota.

Whilst the final separation of Zealandia from Gondwanaland could be viewed as a finite event in time, biological exchange would not have ceased at this point. Opportunities for gene flow would have been reduced, but Zealandia would have been subjected to an ongoing rain of propagules and individuals. As it moved further from Australia, it is likely that the intensity of this rain diminished but the effects would be regionally variable with northern Zealandia

(New Caledonia) remaining closer to Australia than the south (New Zealand), offering different potential colonisation rates. Dispersal rates are likely to have been dependent on changing wind and ocean currents (e.g. initiation of the circumpolar current following separation of Antarctica from Australia (~35 Ma, Veevers, 1991; Sanmartin & Ronquist, 2004), and Antarctica from South America (~28 Ma, Sanmartin *et al.*, 2007), and colonisation rates would be effected by habitat availability and ecological competition (Whittaker & Fernández-Palacios, 2006).

Although far from perfectly described, our understanding of the physical processes influencing the biota of New Zealand continues to improve. What is clear is that the current landscape (its size, shape and topography) is primarily the product of tectonic activity since 25 Ma. The biota too must have developed primarily after that time, whether derived from relict Zealandian lineages or colonists. It is therefore unreasonable to expect an "ancient" (Zealandian) biota in New Zealand developed over >80 million years like that of Australia. Furthermore, if any substantial Zealandian biota, or indeed even a Miocene (<23 million years) biota had survived intact to modern times we would expect not only within-group phylogenetic signal consistent with this but other ecological attributes including clear adaptive radiations and at least some derived interactions (mutualisms and coevolved traits) especially through coevolutionary escalation (Thompson, 2005). But neither of these features of biological complexity are well represented in New Zealand (Didham, 2005), to the extent that even specialised insect pollinators are scarce with most pollination attended to by flies.

Meagre examples of biological complexity include parasitism (scale insects on sourthern beech trees, Harris *et al.*, 2007), animal-plant mutualisms such as the foraging behaviour of the tui honeyeater (*Prosthemadera novaeseelandiae*) on native mistletoes (Loranthaceae) (Robertson *et al.*, 1999), and the pollination of the wood rose (*Dactylanthus taylorii*) by the largely terrestrial short-tailed bat (*Mystacina tuberculata*) (Ecroyd, 1996). However, these appear to be the exceptions that prove the rule. A proposal that some weta (anostostomatid orthopterans) may have a mutualistic relationship with native fruiting plants (Duthie *et al.*, 2006), has been extended to the suggestion that general features of New Zealand fruiting shrubs might be products of coevolution with weta (Burns, 2006). However, this notion is unsubstantiated and numerous lines of reasoning indicate it is unconvincing (Morgan-Richards *et al.*, 2008).

This lack of biological complexity in New Zealand may reflect a lack of observations in some cases, one or more episodes of major biotic disruption or turnover, or simply the youth of the New Zealand biota. Significantly, the pre-Holocene terrestrial fossil record in New Zealand (mostly plants until recently, but see Worthy *et al.*, 2006) indicates a substantial turnover of the biota after the mid-Miocene (Pole, 1994, 2001; Lee *et al.*, 2001). Clearly, from this perspective alone there is little justification in viewing the biota as ancient.

Nevertheless the New Zealand biota does have many peculiarities when viewed from a global perspective. Is this evidence for ancient interactions? One of the most striking is the unusual 'divaricate' growth form typical of many New Zealand woody plants. Divaricate plants, which typically have a wide branching angle, closely interlaced branches and small leaves

concentrated to the interior of the plant are rare elsewhere in the world but are represented in New Zealand by more than 50 species in 27 genera from 22 families or around 10% of native woody plant species (Wardle, 1991). Alternative hypotheses advanced to explain this growth form include adaptation to herbivory by moa, and response to cold, dry Pleistocene climate. Regardless, many divaricates have closely related large leaved relatives, which suggests divarication has evolved during recent geological time rather than over 80 million years (Greenwood & Atkinson, 1977; Lusk, 2002; Howell *et al*., 2002).

Similarly, the existence in New Zealand of the world's largest eagle (*Harpagornis moorei*) indicates adaption to large prey (moa) in the absence of other large (terrestrial mammal) predators. The giant eagle-moa relationship could reasonably be assumed to attest to an ancient adaptive history but instead it appears to have evolved during the Pleistocene (<1.8 Ma) from a small Australian colonist (Bunce *et al.*, 2005). Evolution of *Harpagornis* therefore also nicely demonstrates the rapidity and extent of morphological evolution on an oceanic island rather than testifying to the ancient isolation of New Zealand's fauna. Indeed, from the perspective of the moa, there is as yet no direct evidence (oldest moa bones are late Pliocene, Worthy *et al*., 1991) that their pre-Pleistocene ancestors were giants before the emergence of *Harpagornis*.

Molecular evidence for diversification after the Oligocene "crisis" (Cooper & Cooper, 1995) is not extensive. Early analysis of moa (and wren) diversification indicated that their radiations might date to early Miocene time (Cooper *et al.*, 2001), but more recent analyses indicate the moa radiation is younger and primarily Pliocene (Baker *et al*., 2005). Perhaps this too reflects a late Miocene climate-related assemblage change, as indicated by the plant fossil record. If an extensive extinction phase occurred in the late Miocene in response to rather subtle climate change, we must accept that earlier and more intense environmental perturbations (e.g. submergence of Zealandia) would have had equal or greater impact on the biota. The fact that the signal from earlier extinctions (and colonisations) is obscured by later events should not prevent us from seeking evidence for them. Biologists frequently refer to New Zealand's "turbulent geological history" (e.g. McDowall, 2000; Trewick, 2000*b*; Apte *et al*., 2007; Stevens *et al.*, 2007), but our knowledge of the major phases of Zealandia and New Zealand geophysical history ought to provide the basis for rather more sophisticated hypotheses about which periods were most evolutionarily influential. Major events since separation of Zealandia include K/T asteroid impact, Oligocene submergence, Miocene and Plio-Pleistocene climate change, Pliocene orogenics and Pleistocene volcanics.

II.4 CONCLUSION

To advance our understanding of the evolutionary history of New Zealand we especially need more molecular studies, in an appropriate taxonomic framework linking New Zealand fauna to their counterparts in other parts of the Pacific, Australasia and the world. There are relatively few such studies of terrestrial animals but rather more to date on plant taxa. The presumption that the New Zealand fauna is captive and thus monophyletic is untenable and inappropriate as a starting point if meaningful inferences of biological history are to be made, but suitable sampling allows the prediction of New Zealand monophyly to be examined (e.g. Breitwieser & Ward, 2003; Arensburger *et al*., 2004*a*; Shepherd & Lambert, 2007; Trewick *et al.*, 2008*b*; Pratt *et al.*, 2008). Important obstacles for understanding processes in the formation of the New Zealand biota is the patchy fossil record in New Zealand and Australia and some taxa are not amenable to biogeographic analysis where they are represented by one or few extant lineages (e.g. hihi, Driskell *et al.*, 2007). In some cases it might be impossible to prove 'ancient' origin or disprove recent arrival where a group is underepresented in one or more geographic areas; missing lineages have a major effect on interpretation of phylogenetic trees (Crisp & Cook, 2005). Research should consider the potential perturbations of the New Zealand biota, its gains and losses over the last 80 million years and much more sophisticated approaches to modelling biological and geophysical process need to be developed to better document for New Zealand's past (e.g. Alloway *et al*., 2007). Biologists are increasingly recognising that the New Zealand biota is not a museum of relicts but a dynamic and relatively young evolutionary system (McGlone, 2005; Didham, 2005). Biologists should not set New Zealand apart as a 'continental island' (Cowie & Holland, 2006) but at the same time, New Zealand is certainly no more a 'fly-paper of the Pacific' (McGlone, 2005) than oceanic islands like Hawaii. The challenge for students of New Zealand biogeography will be to explore the evolution of our biota in the context of the wider Zealandian history of the region. It remains that we are not in the enviable position of knowing for certain our land is emergent and is thus entirely populated by immigrants.

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IIIa

Species radiation of Carabid beetles (Broscini: *Mecodema*) in New Zealand*

ABSTRACT

The New Zealand biota has long been viewed as of a Gondwanan origin and age. However, it is increasingly apparent from molecular studies that diversification, and in many cases origination of lineages, postdate the break-up of Gondwana. In particular, studies of many New Zealand plant groups indicate colonization and species radiation in relatively recent times. Fewer examples of extensive species radiation in animals have been documented in detail, and here we report on a species-rich genus of carabid beetles (*Mecodema*). The aim of this study is to date the maximum age of radiation of this genus in New Zealand and infer unique relative divergence rates for the employed mitochondrial gene regions (COI, COII, 16S). Constrained stratigraphic information (emergence of the Chatham Islands) was used to calibrate Bayesian relaxed molecular clock estimates for diversification of *Mecodema* and a comparison with the commonly applied fixed divergence rate of invertebrates (2.3% / Myr) was made. The inferred timings indicate radiation of these beetles no earlier than the mid-Miocene with most speciation being younger, dating to the Plio-Pleistocene. Like in other Carabid studies worldwide the divergence rates of the individual mitochondrial genes studied in *Mecodema* are much lower than the 2.3% / Myr rate, ranging from 0.5% to 1.84%. This study shows that *Mecodema* is an impressive example of a recent insect species radiation in New Zealand and it adds to the increasing phylogenetic evidence indicating that the biology of New Zealand is primarily the result of recent radiation and speciation.

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IIIa.1 INTRODUCTION

The nature of New Zealand has always drawn much interest from biologists as its biotic composition cannot readily be classed as purely oceanic or continental (Daugherty *et al.*, 1993; Trewick & Morgan-Richards, 2009). The landscape is continental in stratigraphic composition and origin, founded on a tectonic fragment separated from Gondwanaland ~80 million years ago (Ma) - referred to as Zealandia (Campbell & Hutching, 2007; Trewick *et al.*, 2007; Landis *et al.*, 2008) - and is widely treated as being continental in its biology (Cowie & Holland, 2006). New Zealand does harbour a distinctive biota including several apparently relic taxa including tuatara (*Sphenodon*), leiopelmatid frogs, and a so called Gondwanan element including weta (Orthoptera), peripatus (Onychophora), southern beech (*Nothofagus*) and kauri (*Agathis australis*) (Cooper & Millener, 1993), but it is also home to many very recent colonists (Fleming 1979; Goldberg *et al.*, 2008; Wallis & Trewick, 2009; Trewick & Gibb, 2010). As a result of this eclectic biotic composition and poorly understood history, New Zealand has been a focus for studies of speciation and colonization (Gillespie & Roderick, 2002). Endemicity at species level is high (Trewick & Morgan-Richards, 2009), comparable with island biota such as Hawaii and the Galapagos (Gibbs, 2006), and it has thus been described as a hot spot for endemism (Daugherty *et al.*, 1993). Endemicity at higher taxonomic levels is however much lower, lower than would be expected from 80 million years (Myr) of isolation implied by geology. Importantly, emphasis on the origin of New Zealand lineages (stem groups) undervalues the much greater evolutionary significance of crown groups in the assembly of the New Zealand biota. A number of studies indicate that an alternative emphasis to the ancient vicariant origin is warranted. The results of molecular studies implicate recent diversification often following long distance dispersal for a diverse set of New Zealand animals and plants (Christidis *et al.*, 1996; Hurr *et al.*, 1999; Trewick, 2000; Chambers *et al.*, 2001; Winkworth *et al.*, 2002; de Queiroz, 2005; Didham, 2005; Knapp *et al.*, 2005; Perrie & Brownsey, 2007; Chapple *et al.*, 2009; Trewick & Gibb, 2010). Additionally it is evident that there is no consensus in the phylogeographic patterns in the New Zealand biota with no consistent pattern among lineage formation, landscape history and distribution of taxa (Goldberg *et al.*, 2008; Wallis & Trewick, 2009).

Following its separation from Gondwanaland between 80 and 62 Ma, the continent of Zealandia was mostly submerged beneath the sea (Campbell & Hutching, 2007; Trewick *et al.*, 2007) and modern New Zealand is primarily the product of tectonic activity initiated ~25 Ma (Landis *et al.*, 2008). How much land persisted in the region prior to this phase is debated, but extreme reduction is certain and this period is thought to have caused a biological crisis and a severe biotic bottleneck (Cooper & Cooper, 1995). Thus, whatever the source of lineages in New Zealand (oversea dispersal or Zealandian survivors), most diversity is unlikely to be older than early Miocene. Subsequent tectonic activity in the Miocene and Pliocene resulted in substantial remodelling of the archipelago and culminated in crustal uplift and orogenesis since the Pliocene (~5 Ma; Kamp, 1992). Biotic turnover since early Miocene time is also evident from the fossil record and this might reflect a shift from tropical to temperate climate

(Lee *et al.*, 2001). Accompanying recognition of the relative youth of New Zealand's biological composition, there is naturally increasing interest in how diversification is distributed through time and space.

Integral to furthering our understanding of the rates and modes of speciation is dating of phylogenetic lineage formation. Despite some criticism (Heads, 2005*a*, 2005*b*) molecular dating with appropriate calibrations provides an empirical approach to estimate timing of past speciation events and phylogeography (Ho & Phillips, 2009). Determining the age of endemic biota has been largely dependent on the use of molecular clock calibrations, and the dating of speciation in New Zealand's plants and animals is especially problematic due to the generally poor fossil record for many lineages. Even where fossils are present, their use requires good time constraint and confidence in their ancestral status for respective extant taxa (Ho & Phillips 2009). Similarly, the use of vicariance events requires confidence, that is usually lacking, in the role of vicariance in lineage formation (Trewick & Gibb, 2010). Therefore biologists have often relied upon generalized molecular clock rates or have used stratigraphic evidence that might correlate to past geophysical events. Many studies (e.g. Trewick, 2000; Zeh *et al.*, 2003; Satoh *et al.*, 2004; Morse & Farrell, 2005; Sole *et al.*, 2005) have, for want of an alternative, estimated divergence times in many invertebrate taxa using a fixed divergence rate of 2.3% per Myr for mitochondrial genes that was inferred for the South American butterfly *Heliconius erato* (Brower, 1994). However, other studies show that mtDNA divergence rates in invertebrates may vary from 0.4– 8.6% / Myr depending on the taxon and the gene-region investigated (e.g. Prüser & Mossakowski, 1998; Nieberding *et al.*, 2004; Quek *et al.*, 2004; Pons *et al.*, 2010; Papadopoulou *et al.*, 2010), so some authors employ a range of rate estimates to calculate divergences inferring a strict molecular clock (e.g. Marek & Kavanaugh, 2005; Neiman *et al.*, 2005; Hogg *et al.*, 2006). Such an approach attempts to accommodate uncertainty about substitution rate by using uncertain calibrations, but assumes that DNA sequences evolve in a clock like manner, even though it is well recognized that molecular evolution is not constant and varies among genes, taxonomic groups and across time (Avise, 2004). Data that do not evolve in a clocklike manner are evidently subject to rate heterogeneity and therefore likely to yield misdating of some or all nodes in a tree if a strict molecular clock approach is applied.

Here we use the earliest possible establishment of an island biota on the Chatham Islands as a conservative calibration for dating diversification of *Mecodema* (Blanchard, 1843) carabid beetles (tribe Broscini). This endemic genus of large, flightless beetles constitutes a prominent species radiation in New Zealand. The Chatham Islands are located approximately 850 km east of New Zealand in the Pacific Ocean (figure 3a.1). It has previously been argued on the basis of the Chathams' shared continental origin with New Zealand that these islands harbour ancient biota since the break up of Gondwana (~70 Ma; Craw 1988). However, geological evidence for a much more youthful origin of the modern Chatham archipelago is compelling

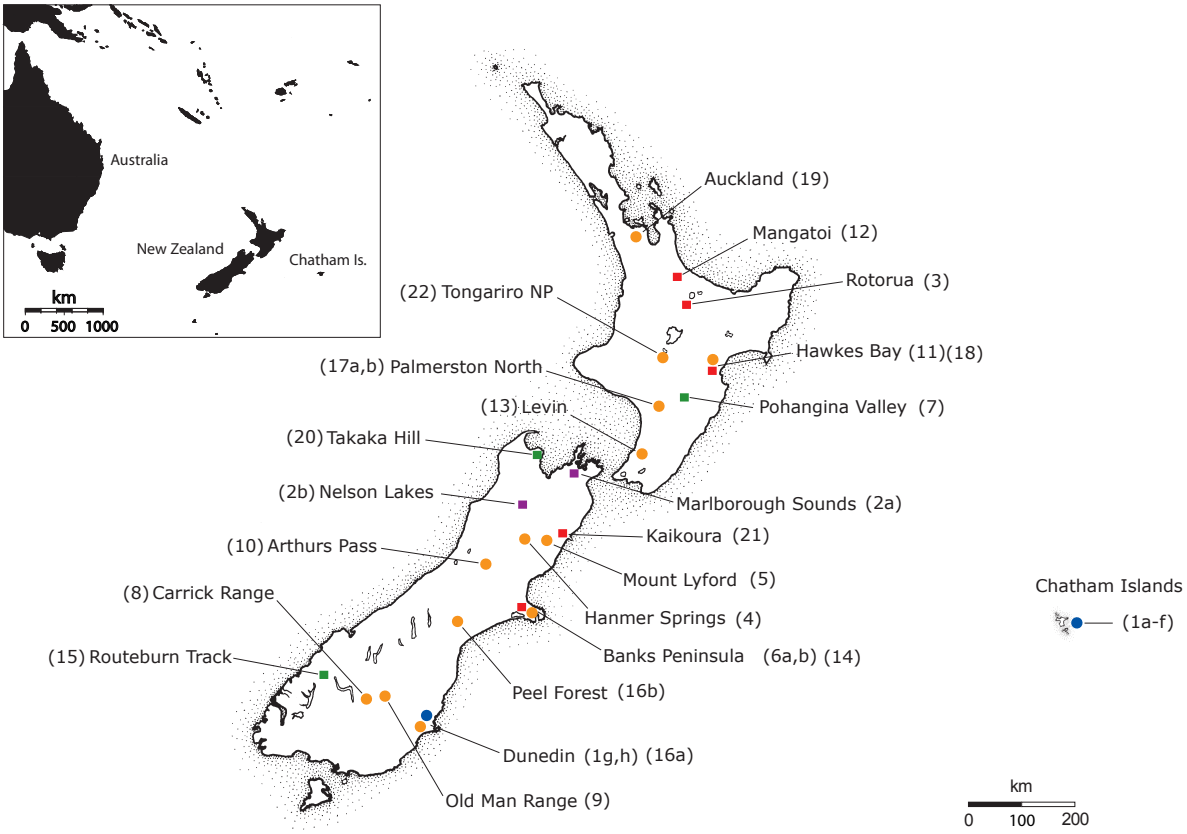

Figure 3a.1 Map of the New Zealand region with sampling locations of *Mecodema* specimens in New Zealand including Chatham Islands. The numbers in parentheses correspond with sample numbers in Table 3a.1 and symbols depicting sampling locations correspond with clades in figures 3a.3, 3a.4a and 3a.4b.

(Campbell, 1998; Campbell *et al.*, 2006) and corroborated by genetic data for many taxa (e.g. insects, Trewick, 2000; plants, Wagstaff & Garnock-Jones, 1998; parakeets, Boon *et al.*, 2001; pigeons, Millener & Powlesland, 2001, Goldberg *et al.*, 2011; cicadas, Arensburger *et al.*, 2004; invertebrates and plants, Paterson *et al.*, 2006; ferns, Shepherd *et al.*, 2009).

We examine the phylogenetic relationship and timing of radiation of the genus *Mecodema* in New Zealand utilising the fact that the genus is represented on the Chatham Islands and use the maximum age of 4 million years for the land surface of the Chathams (Campbell, 1998) as this provides a robust maximum age limit for the timing of colonization of this archipelago by these terrestrial taxa. We also infer unique divergence rates for the mitochondrial genes employed.

IIIa.2 MATERIAL & METHODS

Sampling

The genus *Mecodema* belongs to the tribe Broscini (Carabidae) which has a worldwide distribution but has its main occurrence and greatest diversity in the southern hemisphere (subfamily Nothobroscinae) (Roig-Juňent, 2000). Broscini consists of at least 27 genera, and

the five genera present in New Zealand are endemic and comprise 68 species (Britton, 1949). Adult beetles are relatively slow-moving, nocturnal, flightless (with fused elytra), generally active throughout the year, and usually scarce (Hutchison, 2001). As with other Carabidae, adults and larvae of the New Zealand taxa are predatory. *Mecodema* is a diverse genus with species distributed throughout the New Zealand mainland from alpine to coastal habitats. In contrast, there is a single species (*Mecodema alternans*) on the Chatham Islands. The same species occurs in southeast New Zealand in the vicinity of Dunedin (figure 3a.1). Although *M. alternans* may be better treated as a species complex (Larochelle & Larivière, 2001), no morphological characters have yet been described that distinguish Chatham Island populations from those in Otago (Townsend, 1971).

Of the 57 known *Mecodema* species (after Britton, 1949) 33 specimens from 19 described species and 3 as yet undescribed species (I. Townsend, pers. comm.) were obtained for this study (Table 3a.1). In addition, we included representatives of three other genera of New Zealand Broscini (*Oregus* (Putzeys, 1868), *Diglymma* (Sharp, 1886) and *Metaglymma* (Bates, 1867)) as putative outgroup taxa. The specimens were preserved in 95% ethanol after hand collection in the field, and stored with unique voucher numbers at Massey University, Palmerston North. This sample represents the taxonomic and geographic range of *Mecodema* (see figure 3a.1).

DNA extraction, amplification and sequencing

DNA was extracted from a single leg of each specimen using a salting-out extraction protocol (Sunnucks & Hales, 1996). We employed mitochondrial genes for determining the species level relationships as they have a relatively fast evolutionary rate (Brown *et al.*, 1979) and lack recombination (Olivio *et al.*, 1983). Partial cytochrome oxidase I (COI) and II (COII) and full-length 16S were amplified for all specimens. The COI gene region (776 bp) was amplified using primers C1-J-2195 and L2-N-3014, the COII gene region (547 bp) using primers TL2-J-3037 and C2-N-3661, and 16S (1313 bp) using primers N1-J-12585 and LR-N-13398 (Simon *et al.*, 1994). PCR amplifications were performed in 10µl volumes using Red Hot Taq (ABgene). Purified DNA fragments were treated to cycle sequencing using Big Dye v3.1 terminators under standard conditions on an ABI 377 sequencer (ABI). Sequence identity was confirmed by comparison with published data and checked for nucleotide ambiguities and stop codons in the translation that might indicate the presence of nuclear copies. The sequences will be deposited at Genbank.

Phylogenetic analysis

Sequence reads were verified using Sequencher (Gene Codes Corporation, Ann Arbor, MI, www.genecodes.com) and aligned by eye using Se-Al v2.0a11 (Rambaut, 1996). The tRNA-Leucine between COI and COII was excluded and the resulting three mitochondrial gene regions were analysed separately and in combination. Phylogenetic trees were rooted using *Diglymma* and *Oregus* species, that represent two of the New Zealand Nothobroscina genera

Table 3a.1 List of investigated Broscini samples with sample numbers corresponding to numbers in figures 3a.1 and 3a.3 and species names with sampling locations. (M. = *Mecodema*; Meta. = *Metaglymma*; O. = *Oregus*; D. = *Diglymma*; S.I. = South Island New Zealand; N.I. = North Island New Zealand; BOP = Bay of Plenty).

Sample #	Sample ID	Species	Location
1a	MB 01	*M. alternans*	Chatham Is., South East Is.
1b	MB 02	*M. alternans*	Chatham Is., South East Is.
1c	MB 70	*M. alternans*	Chatham Islands, Mangere Is.
1d	MB 71	*M. alternans*	Chatham Islands, South East Is.
1e	MB 86	*M. alternans*	Chatham Is., Mangere Is.
1f	MB 87	*M. alternans*	Chatham Islands, South East Is.
1g	MB 14	*M. alternans*	S.I., Dunedin, Taieri Mouth
1h	MB 16	*M. alternans*	S.I., Dunedin, Sandfly Bay
2a	MB 79	*M. crenicolle*	S.I.,Marlborough Sounds, Pelorus Bridge, Circle Track
2b	MB 103	*M. crenicolle*	S.I., Nelson Lakes, St. Arnaud Range, Wairau River
3	MB 66	*M. curvidens*	N.I., BOP, Rotorua
4	MB 110	*M. fulgidum*	S.I., Hanmer Springs, Clarence Valley, Mt. Percival
5	MB 91	*M. cf fulgidum*	S.I., Seaward Kaikoura Range, Mt. Lyford
6a	MB 98	*M. howittii*	S.I., Canterbury, Banks Peninsula, Otepatotu
6b	MB 99	*M. howittii*	S.I., Canterbury, Banks Peninsula, Otepatotu
7	MB 63	*M. longicolle*	N.I., Rangitikei, Ruahine Ra., Pohangina Valley, Takapari Rd
8	MB 19	*M. lucidum*	S.I., Otago, Carrick Range
9	MB 11	*M.* nsp.	S.I., Central Otago, Obelisk top, Old Man Range
10	MB 37	*M.* nsp.	S.I., Arthurs Pass NP, Dome
11	MB 51.1	*M.* nsp.	N.I., Hawkes Bay, Havelock North
12	MB 68	*M. occiputale*	N.I., BOP, Mangatoi, Otanewainuku Forest
13	MB 23	*M. cf oconnori*	N.I., Wellington, Levin, Ohou, Browns Bush
14	MB 90	*M. oregoides*	S.I., Akuriri Scenic Res., Port Hills
15	MB 03	*M. rugiceps*	S.I., Fiordland, Lake Harris, Routeborn
16a	MB 45	*M. sculpturatum*	S.I., Dunedin, Ross Reserve
16b	MB 108	*M. sculpturatum*	S.I., Mid Canterbury, Christchurch, Peel Forest
17a	MB 25	*M. simplex*	N.I., Manawatu, Palmerston North, Pahiatua Track
17b	MB 64	*M. simplex*	N.I., Manawatu, Palmerston North
18	MB 50	*M. spinifer*	N.I., Hawkes Bay, Mohi Bush
19	MB 18	*M. spiniferum*	N.I., Auckland, Waitakeres, Arataki
20	MB 96	*M. strictum*	S.I., Nelson, Takaka Hill, Canaan
21	MB 95	*M. sulcatum*	S.I., Kaikoura, Reserve North of Ohau Point
22	MB 69	*M. validum*	N.I., Taupo, Tongariro NP, Whakapapanui Track
	MB 106	*Meta. moniliferum*	S.I., Mid Canterbury, Christchurch, Quail Island
	MB 107	*Meta. moniliferum*	S.I., Mid Canterbury, Christchurch, Quail Island
	MB 13	*O. nsp*	S.I., Otago, Danseys Pass
	MB 41	*O. aereus*	S.I., Dunedin, Morrison St.
	MB 48	*D. clivinoides*	S.I., Seaward Kaikoura Range, Upper Tinline Valley
	MB 31	*D. clivinoides*	S.I., NW Nelson, Heaphy Track

taxonomically closest to *Mecodema* (Roig-Juňent, 2000). The third putative outgroup taxon examined, *Metaglymma*, was not supported as belonging to the outgroup (see below). In some instances replicate species samples were deleted from the dataset to reduce computing time. Partition-homogeneity tests (PHT, Farris *et al.*, 1994) were implemented in PAUP*4.0b10 (Swofford, 1998) with 500 replicates for the combination of the three gene regions to detect significant heterogeneity among the data sets. We conducted neighbor-joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses, as implemented in PAUP* with the combined and individual datasets of the gene regions. NJ and ML analyses were conducted under the respective best fitting nucleotide substitution models. These were selected using the hierarchical Likelihood Ratio Test (hLRT) and the Akaike Information Criterion (AIC) as implemented in Modeltest 3.5 (Posada & Crandall, 1998). All MP analyses used stepwise addition to assemble the starting tree, followed by Tree Bisection Reconnection (TBR) branch swapping with all characters given equal rates. ML analyses were conducted using a heuristic search with a NJ starting tree and TBR branch swapping. Bootstrapping utilized 1000 replicates to test the stability of tree topology under all optimality criteria (NJ, MP and ML). Gaps in the 16S sequence alignments were treated as missing data. Genetic distances were calculated using PAUP* 4.0b10 (Swofford, 1998). Nucleotide diversity and frequencies were estimated in MacClade 4.0 (Maddison & Maddison, 2001). MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) was used to implement Bayesian analysis with the concatenated dataset applying a GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites. The same model was applied to the three partitions (COI, COII and 16S) with rates and nucleotide frequencies for each gene unlinked. Analyses with MrBayes used four independent Markov Chain Monte Carlo (MCMC) runs for two million generations with a burn-in of 25%. Resulting posterior probabilities on the nodes were recorded.

We used SplitsTree 4.0 (Huson & Bryant, 2006) to construct unrooted networks under various models for the different genes to visualise conflicting signal within the dataset for alternative topologies (Holland *et al.*, 2004). We implemented Shimodaira-Hasegawa tests in PAUP*, and used these, along with bootstrap scores to assess support for alternative topologies.

Molecular dating

To assess whether the genes in our sample evolved according to a molecular clock hypothesis we reconstructed phylogenies for all datasets under ML-criterion and the best fitting models with and without a molecular clock enforced. Likelihood-Ratio-Tests (LRT) were conducted for each data set using $\Delta = 2*(-\ln L_1-(-\ln L_0))$ (Felsenstein, 1988) and the χ^2 – test to compare the different hypotheses.

For molecular dating we used the software BEAST v1.4.8 (Drummond & Rambaut, 2007). All analyses were conducted with a Yule tree prior and a UPGMA starting tree under the GTR+I+Γ model of nucleotide substitution. The MCMC was run for 10 million generations,

sampling every 1000th step after a discarded burn-in of 1000 sampled trees. Each analysis was run 4 times. The program Tracer v1.4 (Drummond & Rambaut, 2007) was used to summarize posterior distributions of all parameters in question, to verify convergence of the MCMC and to estimate Effective Sample Sizes (ESS). Posterior distributions of all parameters were estimated from the combined posterior distributions of all runs. The program FigTree v1.1.2 (Drummond & Rambaut, 2007) was used to visualize the reconstructed phylogenies.

To evaluate whether COI, COII and 16S evolve at the approximately same mean rate we first estimated the ages of nodes B, I and J (figure 3a.2) for each of the genes assuming a strict molecular clock and a substitution rate of 0.0075 subst/site/myrs (1.5% pairwise divergence per million years, a rate derived from analyses of multiple invertebrate taxa; Quek *et al.*, 2004). We then compared age estimates for these nodes in the COI, COII and 16S phylogenies and found that age estimates for 16S differed significantly from those for COI and COII. We used the posterior distribution of the age of the root in the COI phylogeny as a prior to calibrate the age of the root in COII and 16S phylogenies and estimate mean substitution rates for both genes under a strict molecular clock.

To obtain estimates for the age of species radiation events within *Mecodema* we used the concatenated dataset of all three genes and a number of different calibration strategies. To allow for rate variation along branches we assumed an uncorrelated lognormal distribution of substitution rates along the phylogenies. First we estimated divergence times on the complete dataset consisting of all three genes using the 1.5% divergence rate (Quek *et al.*, 2004) for COI as a conservative approach. To do this we used the posterior distribution of the age of the root in the COI phylogeny to calibrate the complete dataset phylogeny. In a second step, we estimated the age of the same radiation event using a geological calibration and two different colonization scenarios. In both cases the split between Chatham Island *M. alternans* and its closest relative on mainland New Zealand (NODE B) was used as the calibration point for these estimations. In

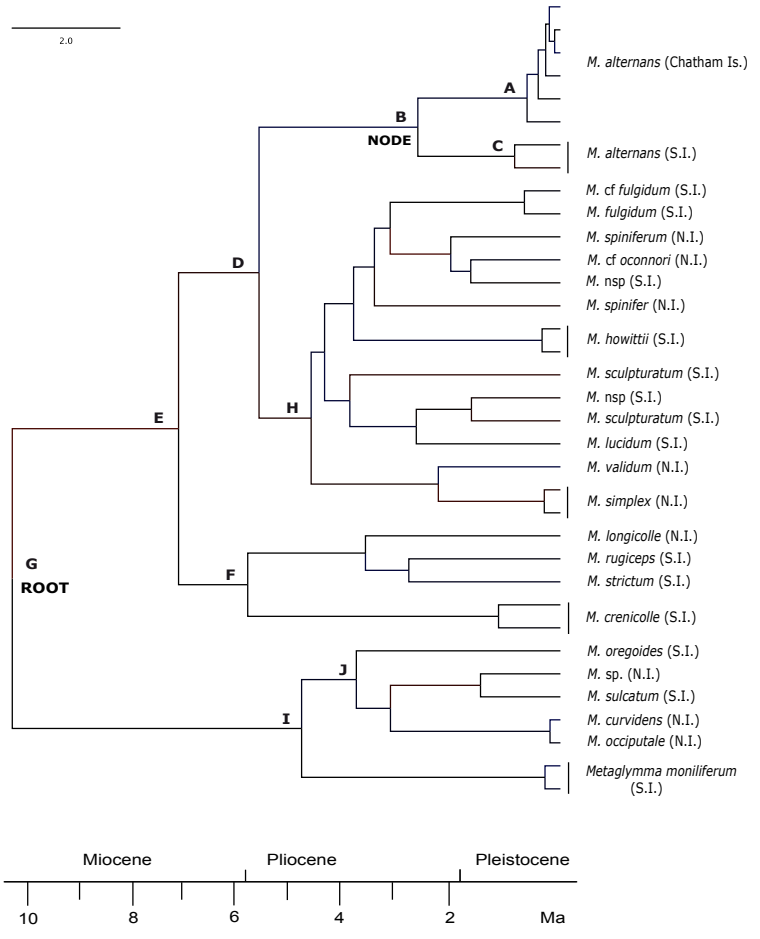

Figure 3a.2: Ultrametric tree of the concatenated dataset (COI, COII and 16S) generated with BEAST v1.4.8; letters on nodes correspond to letters inTable 3a.5.

the first scenario we assumed a normal distribution for the age around a calibration value of 3 Myr, derived from the maximum age for the Chatham Islands land surface (Campbell *et al.*, 2006; Campbell & Hutching, 2007) and assuming that colonization was most likely soon after emergence of the islands. In the second scenario we assumed a uniform distribution of 0.01-4.01 Myr for the age of the calibration point, assuming an equal likelihood of colonization at any time after formation of the islands.

For comparison we estimated divergence times using the Bayesian Relaxed Molecular Clock (BRMC) (Thorne *et al.*, 1998; Kishino *et al.*, 2001; Thorne & Kishino, 2002). Again the split between the Chatham Island species *M. alternans* and its closest relative on mainland New Zealand (*M. alternans*) was used as the calibration point for the calculations. In BRMC the calculation was run with this node set to lower and upper bounds (1-4 Myr) and it was repeated under the assumption of an ancient split between the Chatham Islands and New Zealand (60-80 Myr) according to the requirements of the program and the available priors.

For the BRMC method (Thorne *et al.*, 1998) we employed the program multidivtime as implemented in the T3 package (Thorian Time Traveller) (Yang, 2003). After having identified the ML topology of the dataset the first step was to use the program baseml of the PAML package version 3.13 (Yang, 1997) to calculate the ML parameters of the F84+Γ substitution model (http://abacus.gene.ucl.ac.uk/software/paml.html). The branch lengths of the ML topology and the corresponding variance-covariance matrix were calculated using the program Estbnew (ftp://abacus.gene.ucl.ac.uk/pub/T3/). The mean posterior divergence times on the nodes with standard deviations were taken from the variance-covariance matrix by running a Markov chain Monte Carlo in the program multidivtime. The Markov chain was sampled 10,000 times every 100 cycles after a burn-in stage of 100,000 cycles. We used an early Miocene age (20 Myr SD = 10 Myr) prior for the expected number of time units between tip and root of the tree to take into account the probable severe bottleneck the New Zealand biota experienced during the late Oligocene and the following rapid radiation. Another prior of 200 Myr was selected to fall between the advent of the first known Coleoptera (~280 Mya, early Permian) (Beutel, 2003) and the first known Carabidae (~ 160 Myr) (Grimaldi & Engel, 2005) for the highest possible number of time units between tip and root as required by the program. The gamma distribution of the rate at the root and the Brownian motion constant were obtained from the median branch length of the tree excluding the outgroup as recommended in the manual. Node times, as mentioned above, were constrained to 1-4 Myr and 60-80 Myr respectively, each analysis was run four times to verify the results and a mean for each prior was calculated.

Additionally we used the maximum ML – distances from the individual gene phylogenies in conjunction with the most widely cited divergence rate for mitochondrial genes in invertebrates (2.3% pairwise divergence/Myr; Brower, 1994) for estimating the age of species radiation events within the genus *Mecodema*.

IIIa.3 RESULTS

The complete alignment of the three gene regions comprising 39 specimens (including 4 outgroup specimens) was 2211bp and had an overall A-T bias of 75.3%. All three individual genes display the typical A-T bias of insect mitochondrial DNA averaging 75%. For detailed statistics of the gene regions studied see Tables 3a.2.

Phylogenetics

The partition homogeneity test (PHT) revealed no significant heterogeneity of lineage partitioning among the data sets (p = 0.866), suggesting that the three mitochondrial gene regions can be concatenated. The GTR+I+Γ model of nucleotide substitution was identified as the best fitting model by both the hLRT and the AIC as implemented in Modeltest 3.5.

The different log likelihood scores for the calculations with and without a molecular clock enforced and the parameters of the likelihood ratio test (LRT) are shown in Table 3a.3. The concatenated dataset supported a single topology in all analyses and PAUP* generated a single phylogram under the chosen ML-model (figure 3a.3). To reduce computing time we excluded the outgroup taxa and nine ingroup taxon replicates from the dataset for bootstrap resampling using ML. Maximum Likelihood bootstrap scores (1000 replicates) for the main clades were in excess of 90% (figure 3a.3) except for one node separating clades □ and ○ / ● which showed weak support in all analyses. This node was then collapsed in Bayesian tree reconstruction using a GTR+I+Γ model, but apart from this difference the resulting consensus tree showed the same overall topology (figure 3a.2). The same composition of taxa and clades were present in the neighbornet-graphs generated in Splitstree

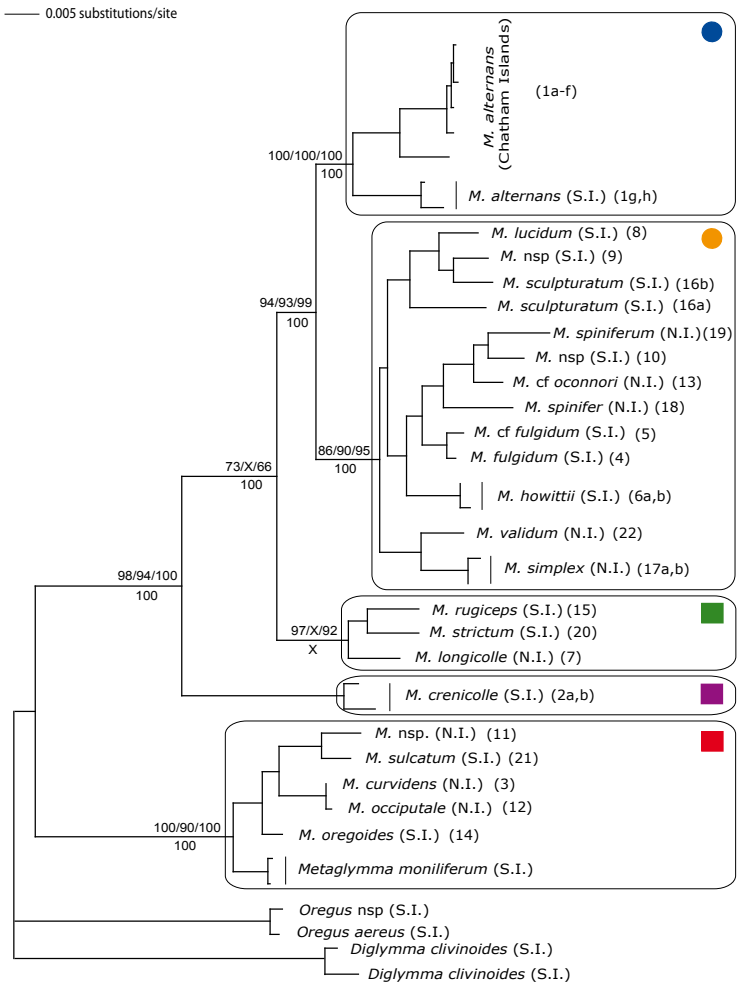

Figure 3a.3: Maximum Likelihood tree based on the concatenated dataset (COI, COII and 16S) with Bootstrap values for the major nodes (NJ/MP/ML above branches; MrBayes below). X denotes no value was available at node for a given optimality criterion. Code numbers at branch tips correspond to those in figure 3a.1 and Table 3a.1; symbols denote species clades and correspond with symbols in figure 3a.1.

Table 3a.2 Summarized statistics of genes excluding outgroup under ML criterion.

Gene	No of taxa	Sequence length (bp)	Variable sites	Ti/Tv	CI	RI	RC	A-T content (%)
COI	35	776	356 (45.9%)	1.68	0.62	0.79	0.49	73.0
COII	35	547	328 (59.9%)	1.07	0.57	0.72	0.41	77.0
16S	35	769	130 (16.9%)	0.82	0.65	0.82	0.54	77.3
COI-16S	35	2092	880 (39.8%)	1.30	0.59	0.75	0.45	75.3

Table 3a.3 Likelihood ratio test results for different genes. Shown are the likelihood values for the different datasets, depending on whether a molecular clock was enforced during a ML heuristic search or not and their p-values. [Combined Data = COI+COII+16S+outgroup; +out = with outgroup included; -out = excluding outgroup; x = evolves not clocklike]

Gene	$-\ln L_1$	$-\ln L_0$ (mol. clock enf.)	Δ [2*-lnL_1-(-lnL_0)]	df (N-2)	p-value (χ^2)	clocklike
COI (+out)	3716.8954	3750.5650	67.34	37	0.001674	x
COII (+out)	3116.8961	3160.3666	86.94	37	$6.724*10^{-6}$	x
16S (-out&reps)	2128.9691	2145.0297	32.12	29	0.3146	√
Combined Data	9739.6551	9782.4390	85.57	37	$1.021*10^{-5}$	x

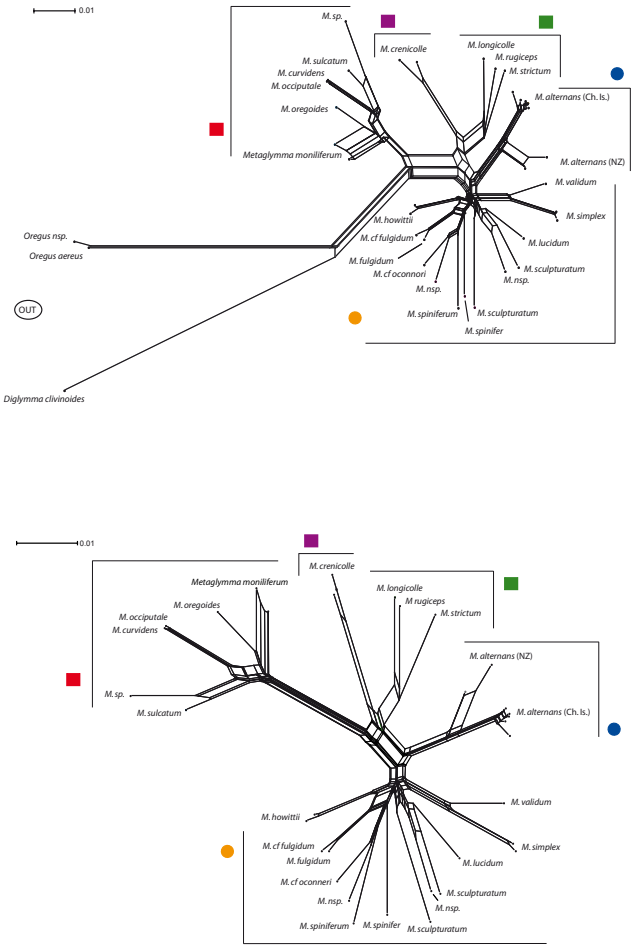

Figure 3a.4a: Splitstree network based on the concatenated dataset (COI, COII and 16S) with outgroup; (b) Splitstree network based on the concatenated dataset (COI, COII and 16S) without outgroup.

(figures 3a.4a & b), indicating that minor inconsistencies within the *Mecodema* ingroup are not the product of inappropriate outgroup selection. *Mecodema* is apparently paraphyletic with respect to *Metaglymma*, as the latter groups within the *Mecodema* complex throughout all datasets and analyses in the study (figures 3a.2 and 3a.3). This phylogenetic position contradicts the current taxonomical classification and needs to be investigated further. Despite a good level of resolution in the mitochondrial DNA gene trees, there is little evidence for spatial correlation of clades with modern terrain of New Zealand. There is no grouping of species in specific North / South Island species or in specific lineages correlating to landscapes. There does, however, appear to be support for the species groupings proposed by Larochelle & Larivière (2001).

Age estimation

Genetic Divergence in Mecodema

Three widely used mitochondrial gene regions were employed to gauge the scale of genetic diversity among the *Mecodema* specimens. Overall we observed relatively low genetic distances among species of *Mecodema* with a maximum ML-distance of 17.9% in COII (COI: 16.1%, 16S: 5.3%). Using these distance data and the divergence rate of 2.3%/ Myr, a strict molecular clock approach gives relatively low age estimates, ranging from 2.29 Myr in 16S to 7.79 Myr in COII, for the ROOT (Table 3a.4). In general these age estimates are lower than the ages inferred using a relaxed molecular clock calculation. For 16S values of the NODE and ROOT were obtained that were less than half those inferred using COII, reflecting the comparatively low proportion of variable sites in this gene (16.9%). The disparity in genetic distances and thus inferred ages highlights an important source of uncertainty when using a uniform divergence rate to estimate the age of lineages with genes that evolve at different rates and do not have a clocklike mode of evolution.

Table 3a.4: Estimated divergence times for the NODE and the ROOT of the different mitochondrial genes using the strict molecular clock approach (2.3%/Myr) with maximal genetic ML-distances and BEAST (95% HPD intervall is given in parentheses).

	Strict [max. ML-distance]		BEAST (normal of 3 Myr)		BEAST (uniform of 0.01-4.01 Myr)	
	NODE	ROOT	NODE	ROOT	NODE	ROOT
COI	1.42 [3.30%]	6.89 [15.9%]	3.01 (1.92 – 4.12)	11.36 (7.89 – 15.64)	3.03 (1.84 – 4.49)	11.35 (7.71 – 15.60)
COII	1.18 [2.70%]	7.79 [17.93%]	3.05 (1.91 – 4.25)	10.81 (6.40 – 16.29)	3.13 (1.77 – 4.15)	10.90 (6.54 – 16.66)
16S	0.45 [1.03%]	2.29 [5.27%]	2.71 (1.63 – 3.95)	9.82 (6.12 – 15.01)	2.31 (1.02 – 4.12)	9.41 (5.64 – 14.56)
COI-16S	0.97 [2.20%]	4.77 [10.96%]	2.64 (1.70 – 3.68)	10.16 (6.93 – 13.85)	2.45 (1.33 – 3.91)	9.85 (6.45 – 14.11)

Table 3a.5: BEAST time estimates for nodes of the concatenated dataset and 95% HPD in parentheses (with normal prior distribution). Letters correspond to letters in figure 3a.2, with bold letters denoting the NODE (B) and the ROOT (G).

Node #	Time estimates (BEAST) in Myr (95% HPD)
A	0.62 (0.30 - 1.10)
B	**2.64 (1.70 – 3.68)**
C	0.85 (0.32 – 1.62)
D	5.58 (3.88 – 7.56)
E	7.08 (4.78 – 9.67)
F	5.79 (x)
G	**10.16 (6.93 – 13.85)**
H	4.62 (3.23 – 6.32)
I	4.80 (3.04 – 7.31)
J	3.78 (2.32 – 5.74)

Table 3a.6: Estimated divergence times for the different mitochondrial genes in *Mecodema*, using the BRMC approach (multidivtime) and BEAST (standard deviation and 95% HP are given in parentheses).

	BRMC (constraint 1-4 Myr)		BEAST (normal of 3 Myr)	
	Node	Root	Node	Root
COI	2.64 (SD=0.80)	8.48 (SD=3.82)	3.01 (1.92 – 4.12)	11.36 (7.89 – 15.64)
COII	2.63 (SD=0.80)	9.70 (SD=3.45)	3.05 (1.91 – 4.25)	10.81 (6.40 – 16.29)
16S	2.24 (SD=0.82)	11.90 (SD=4.94)	2.71 (1.63 – 3.95)	9.82 (6.12 – 15.01)
COI-16S	3.05 (SD=0.66)	12.52 (SD=3.57)	2.64 (1.70 – 3.68)	10.16 (6.93 – 13.85)

Relaxed clock calibrations

The divergence times calculated using the software BEAST v1.4.8 with the set priors for the NODE being 3 Myr (normal distribution) or 0.01-4.01 Myr (uniform distribution) are quite similar. The ROOT is estimated at a maximum age of 10.16 Myr and the NODE at 2.64 Myr for the concatenated dataset with the normal distribution prior and at 9.85 Myr and 2.45 Myr respectively with the uniform prior (Table 3a.4). When estimated for the separate genes the resulting ages fall into the same range (Table 3a.4). The time estimates for principal nodes in the tree of the concatenated data (figure 3a.2) are shown in Table 3a.5.

For the BRMC approach we set lower and upper bounds of 1 and 4 Myr respectively for the NODE as required by the program. The time estimates for the NODE in the different genes varied between 2.24 and 3.05 Myr. The estimated ROOT age ranged from 8.48 Myr with COI up to 12.52 Myr for the concatenated data, but the mean values had overlapping standard deviations (Table 3a.6). The time estimates for the individual nodes of the tree based on the concatenated dataset are not shown. We also calculated the ages with a constraint of 60 – 80 Myr for the NODE (data not shown) but the results were not plausible as the ages suggest an establishment of *Mecodema* in New Zealand approximately 85 Ma and the split to the Chatham Islands at 64 Ma which is inconsistent with the low values of maximum ML distances between the taxa and the geological evidence. Time estimates for the root and the Chatham Island node for all genes and both Bayesian methods are given in Table 3a.6.

Finally, divergence rates for the three mitochondrial gene fragments in *Mecodema* were estimated using the program BEAST v1.4.8. The average rates estimated vary among the mitochondrial gene regions. Using the normal distribution as a prior, we found COII to have the highest rate (1.84% / Myr), COI slightly less (1.18% / Myr) and 16S the lowest (0.50% / Myr) (figure 3a.5). Similar results for the rates were obtained when calculated under the uniform distribution prior (figures 3a.6 a-c).

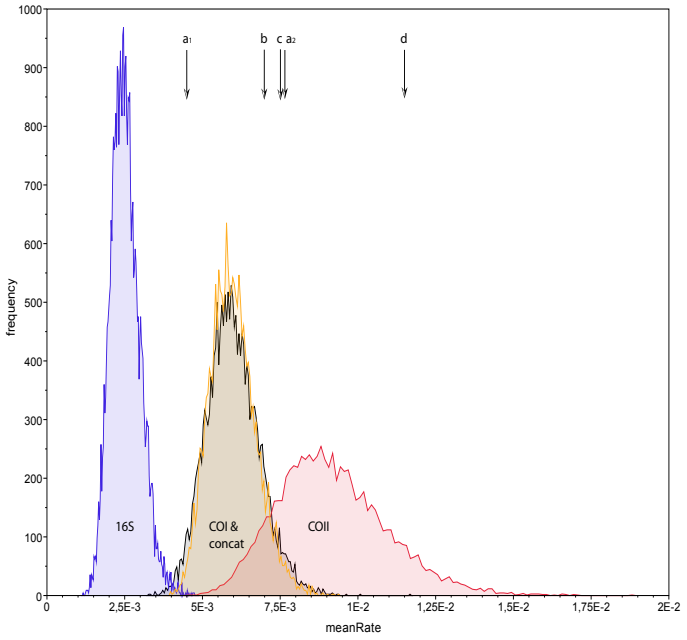

Figure 3a.5: Tracer v1.4 output file showing the distribution of divergence rates inferred for each of the separate genes and the combined dataset. COI and the combined dataset have rate distribution that overlap almost entirely. Arrows indicate invertebrate substitution rates from the literature: (a) Gómez-Zurita *et al.*, 2000: (a1) COII, (a2) 16S; (b) Knowlton & Weigt, 1998, COI; (c) Queck *et al.*, 2004, COI; (d) Brower, 1994, COI.

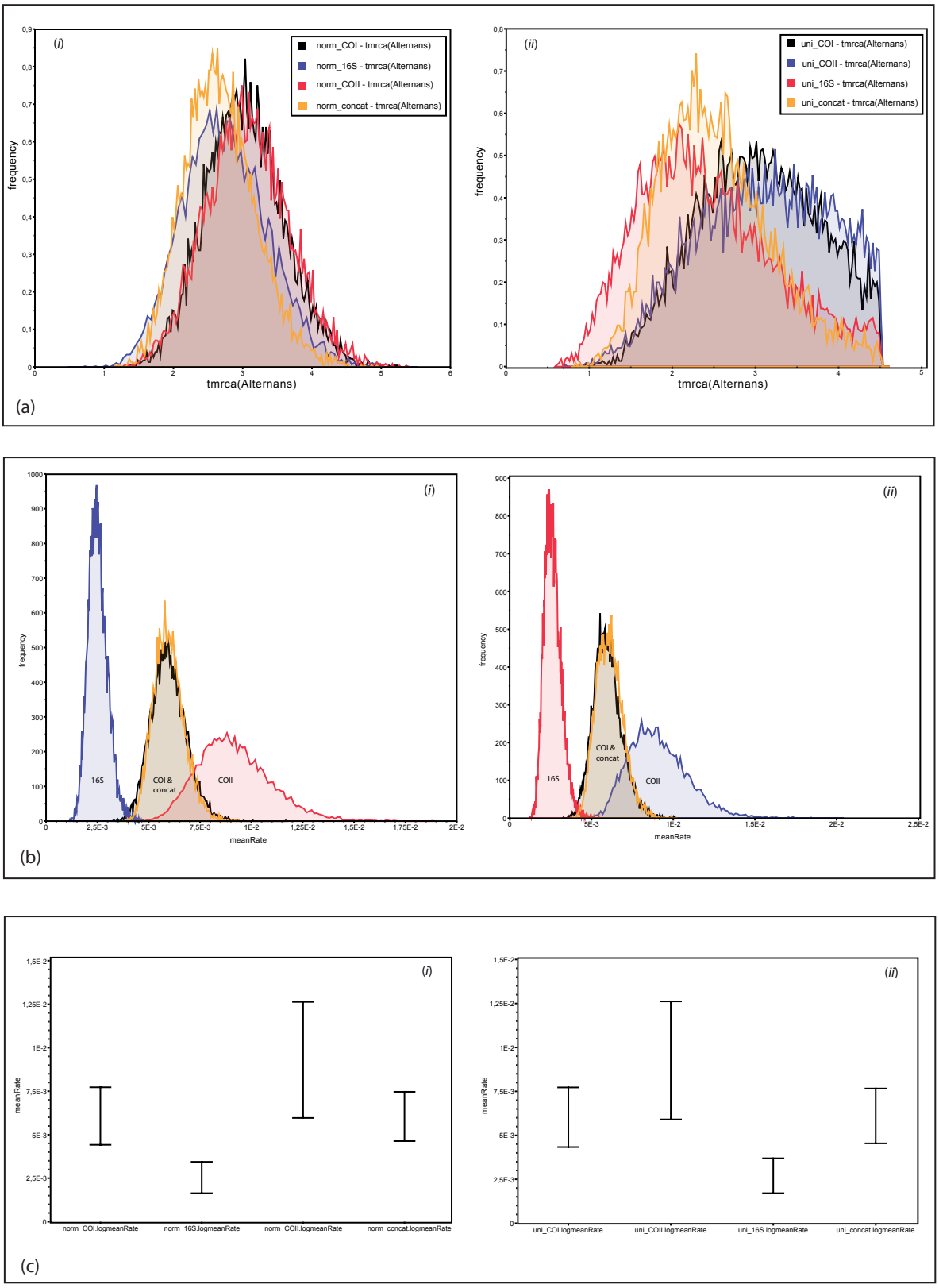

Figure 3a.6: Comparison of Tracer v1.4 output files from Bayesian MCMC runs in BEAST for the genes used in this study and for the two different prior settings for calibration;
(a) Combined Tracer files for the age distribution of the node (Alternans) for a normal distribution prior *(i)* and a uniform prior *(ii)*;
(b) Combined Tacer files for the mean calculated rates with a normal distribution prior *(i)* [see also figure 3.5] and a uniform distribution *(ii)*;
(c) Combined Tracer files depicting the deviation of the mean rates for the different genes, *(i)* for a normal distribution and *(ii)* a uniform distribution of the prior.

IIIa.4 DISCUSSION

In this study we explored the pattern and depth of species diversity in the beetle genus *Mecodema*, which was sampled broadly across known species subgroups (Larochelle & Larivière, 2001) and geographic range in mainland New Zealand and the Chatham Islands. The best supported trees come from the combined data set, although some species-level relationships remain unresolved, even with data from three genes. Most clades are stable across analyses and are largely consistent with the taxonomic groupings proposed by Britton (1949) and Larochelle & Larivière (2001). The failure of these mtDNA sequence data to resolve all species-level relationships in *Mecodema* reflects the very low levels of DNA sequence divergence that we encounter among some species (e.g. maximum of 3.6 % between species of clade ●). This observation in itself suggests recent speciation, which cannot be readily dismissed as oversplitting, as the fine-scale taxonomic subdivision of *Mecodema* is based on sound morphological and ecological differences. *Mecodema* diversity is not simply allopatric, with many species existing in sympatry with congenerics. In addition *Mecodema* spp. are also commonly found with other carabids (e.g. *Megadromus* (Motschulsky, 1866)), which implies additional competitive pressure (Hutchison, 2001), and fine scale ecological delimitation. Thus, high species diversity in *Mecodema* might well be the product of, at least in part, adaptive radiation. A striking example is that of *Metaglymma moniliferum* (Bates, 1867), which by virtue of its distinct morphology has been classified in a separate genus, but is probably better treated as an ecologically specialized *Mecodema*. *Metaglymma* may, following further morphological and genetic investigations, be included in *Mecodema*. The close relationship between *Metaglymma* and *Mecodema*, compared to the two other outgroup genera included in this study (*Diglymma* and *Oregus*) is consistent with the degree of morphological differences among them (Roig-Juňent, 2000). Additional sampling of taxa and populations is necessary to interpret phylogeographic and taxonomic patterns in detail, but the current level of sampling is sufficient for the purposes of gauging the maximum age of species radiation.

We found that substitution rates of mitochondrial genes in *Mecodema* differ among genes and that genes are not clocklike in the way they evolve. Therefore the use of a general invertebrate divergence rate is evidently not appropriate for the genus *Mecodema*, as it does not accommodate the extent of rate variation among genes.

Bayesian relaxed clock methods on the other hand allow rates to vary among lineages although accuracy might still be influenced by the setting of priors, reliability of calibration points (Perez-Losada *et al.*, 2004), rate-heterogeneity and inadequate models (Roger & Hug, 2006). This could just as much lead to wrong age estimates especially if good calibration points, such as fossils or reliable geological dates, are missing. Although it exists an extensive invertebrate fossil record for some parts of the world (Grimaldi & Engel, 2005) it is not simple to incorporate these findings into studies as they might still not give a good calibration. The ideal fossil calibration would consist of accurately dated fossils from closely above and below

the questionable split (Hillis *et al.*, 1996). In most cases however the absence of suitable fossil or geological calibration points simply compromises accuracy of date estimation, and New Zealand is typical of such situations that lack reliable calibration points. Even when geological events are apparently available it is generally difficult to be confident about their resolving power. One fairly well studied instance is the rise of the Isthmus of Panama between North and South America that sundered the Pacific and Atlantic Oceans and their marine biota. Geological date estimates imply that lineages of marine shrimps (Knowlton & Weigt, 1998), echinoderms (Lessios *et al.*, 2001) and molluscs (Marko, 2002) were completely separated approximately 3 Ma by the Isthmus, but it is not known how rapidly this happened or when exactly the total isolation of the populations and species on either side occurred. The same is evident for other much studied systems including the Hawaiian Islands (see for example Fleischer *et al.*, 1998; Roderick & Gillespie, 1998). It is possible to date the age of the islands quite well even if it is not known exactly when the founder population actually arrived on the new archipelago (Shaw, 2002; Jordan *et al.*, 2003), although some inferences about this might be drawn from estimation of speciation rates were sampling is sufficient (Nee, 2004; Mendelson & Shaw, 2005). This and the differences in rates of molecular evolution between genes and taxa are obvious problems when trying to apply molecular clock estimates to date speciation or colonization events. However, the strength of island studies is the given upper bound for the age of the biota. Nevertheless colonization of the Chatham Islands occurring substantially later than the emergence of the islands could lead to underestimation of the age of radiation and thus overestimation of rates of molecular evolution. The reverse would be the case if the age of the calibration points were significantly underestimated (i.e. Chatham Islands are older). However, the age of land surfaces on the Chatham Islands is now remarkably well constraint by stratigraphic and fossil evidence (Campbell *et al.*, 2006). In ideal circumstances the calibration point would be deeper in the tree, so that older ages were not inferred from recent events. Though, the total scale of sequence divergence and thus plausible age range is in geological terms narrow.

In this study the inferred mitochondrial divergence rates are consistently lower than the most commonly cited rate for invertebrates (2.3 %/Myr). The gene regions we studied show mean divergence rates between 0.50 and 1.84 %/Myr which would result in the inference of much higher divergence times if applied with strict molecular clock calculations. Not surprisingly, given their respective structure and function, the protein coding genes COI and COII are the most similar in their divergence rates (1.18%/Myr and 1.84%/Myr respectively), while 16S appears to evolve much more slowly (0.50%/Myr). These estimates for *Mecodema* are consistent with results for mitochondrial genes in other beetle genera that generally indicate divergence rates lower than 2.3%/Myr (e.g. Prüser & Mossakowski, 1998; Gómez-Zurita *et al.*, 2000; Pons *et al.*, 2010; Papadopoulou *et al.*, 2010). In addition, the tendency for timing of recent evolutionary events to be over-estimated due to short-term measurements of mutation rates rather than long-term fixed rates (Ho *et al.*, 2005) might also lead to inferred timing of speciation for *Mecodema* being too high. Because this problem mainly applies to measurements within the past 1-2 Myr

(usually below species level), it could affect the estimations for Chatham Islands' biota and might, in the given case, imply younger ages and thus even lower true substitution rates than those inferred here.

Despite the perception that New Zealand is an ancient continent with an ancient biota, diversity is increasingly shown to be the product of recent speciation congruent with Plio-Pleistocene climate fluctuations and tectonic activity since the mid-Miocene (Cooper & Millener, 1993; Cande & Stock, 2004). Speciation of plants (e.g. Meudt & Simpson, 2006) and animals including vertebrates (e.g. Baker *et al.*, 2005; Bunce *et al.*, 2005) often correlates with relatively recent but profound environmental changes and diversification. For *Mecodema* we used a conservative maximum possible colonization date of 4 Myr for the Chatham Islands and found that radiation of *Mecodema* beetles crown group is unlikely to be older than mid-Miocene with most of the lineage formation in the Pliocene and Pleistocene. This relatively shallow radiation is therefore consistent with the timing of radiations inferred for several New Zealand invertebrates (e.g. cicada, Ahrensburger *et al.*, 2004; weta, Morgan-Richards *et al.*, 2001, Trewick & Morgan-Richards, 2005; cockroach, Chinn & Gemmel, 2004; isopod, McGaughran *et al.*, 2006), vertebrates (galaxiid fish, Waters & Wallis, 2001, Burridge *et al.*, 2008) and plants (buttercup, Lockhart *et al.*, 2001; *Pachycladon*, Heenan & Mitchell, 2003).

The timing of arrival of *Mecodema* or its most recent ancestor in New Zealand cannot, however, be determined from these data. The lineage (stem group) might date back to the separation of Zealandia from Gondwana, having persisted despite marine transgression before the Miocene, or it may have arrived in New Zealand in the Miocene as additional land became available. The fact that this genus and other large flightless insects are present on the Chatham Islands demonstrates that such colonisation oversea is possible. Regardless of the timing and mode of origination in New Zealand, the more important evolutionary feature of this and other groups, with respect to the assembly of the native biota, is the diversification of the crown group. Evidently a few million years have been sufficient to result in a complex ecosytem comprising not simply allopatric subunits but an array of sympatric species with overlapping ecologies. A long geological history is not required. While it has been predicted that intense phylogeographic structuring and speciation dating to the Plio-Pleistocene might be observed more frequently in naturally subdivided alpine conditions than in lowland forests (Trewick *et al.*, 2000), *Mecodema* speciation appears to provide an example where diversification has proceeded across space and into diverse habitats, from coast to above the treeline. Future work on the detailed ecology of these species will be instrumental in demonstrating the mechanisms of diversification (e.g. Sota & Ishikawa, 2004).

We cannot determine from the present data when after emergence of the Chatham Islands *Mecodema alternans* arrived, therefore the age estimates in this study can be considered maximal. Inferred rates of molecular evolution that are lower than comparable estimates and lack of taxonomic distinction of mainland and Chatham populations are consistent with the possibility that the Chatham Islands were colonized late in their history. If so, then the entire

Mecodema radiation could be even younger than we are currently able to demonstrate, with a larger proportion of speciation since the late Pliocene. Further resolution should be possible as time constraints on the development of the New Zealand landscape and habitats improve (Trewick & Morgan-Richards, 2009), but already it is evident that *Mecodema* is an impressive example of recent insect species radiation in New Zealand. Increasingly, synthesis of phylogenetic, ecological and taxonomic evidence indicates that the biology of New Zealand is primarily the story of recent adaptation and speciation (Goldberg *et al.*, 2008, Wallis & Trewick, 2009).

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IIIb

Timing of a carabid beetle (Carabidae: Broscini) radiation in relation to land formation in New Zealand

ABSTRACT

In recent years molecular data in combination with molecular clock approaches is widely applied to estimate the timing of species formation and diversification. Although these approaches have their limitations due to availability or the quality of used priors, they are useful tools to test for congruence of phylogenetic and biogeographic prediction. In the present study this approach is applied to test for congruence between the timing of species formation of the species rich genus *Mecodema* within New Zealand in relation to a very youthful land area in southern North Island of New Zealand. Mitochondrial DNA (cytochrome oxidase I) was used with the previously calculated substitution rate for this gene in *Mecodema* to investigate the phylogenetic relationships and timing of radiation within this genus using a molecular clock approach. The results were then compared to the timing of landscape formation of the lower North Island of New Zealand. The inferred timing indicates most of the speciation in these beetles happened in the last 5 Myr with several North Island / South Island splits between sister taxa within this time frame. This includs taxa that can now be found in areas that have only emerged above sea level in recent geological times. These results suggest that there is a general overestimation from genetic calibration of lineage splits of taxa that were sampled in the young landscape of southern North Island. This implies that the molecular substitution rate applied to these data is an underestimate, therefore resulting in over estimation of lineage ages in *Mecodema*.

IIIb.1 INTRODUCTION

Understanding the timing of speciation and therefore the rates and modes of diversification has been advanced by the application of genetic data and molecular clock tools (e.g. Knapp *et al.*, 2005; Buckley & Simon, 2007; Marshall *et al.*, 2008). The promise of detailed information about how biotic diversity develops is, however, dependent on the quality of taxon and data sampling, calibration tools and calibration information. In no case are all these components perfect for reconstruction of exact biogeographic history, instead hypotheses can be developed and tested. This follows standard scientific method whereby observation via inductive reasoning yields hypotheses that can be tested against further data resulting in the interactive development of improved hypothesis (see Crisp *et al.*, 2011).

Here, this approach is applied to the estimation of the timing of a radiation of New Zealand beetles. The biota of New Zealand is, in evolutionary terms, a dynamic and relatively young system (McGlone, 2005; Goldberg *et al.*, 2008; Wallis & Trewick, 2009). Following its separation from Gondwanaland (83 Ma), most if not all of the continent of Zealandia sunk beneath the sea (Pole, 1994; Waters & Craw, 2006; Trewick *et al.*, 2007; Landis *et al.*, 2008). Present day New Zealand, which is a small emergent part of Zealandia, is mainly the product of tectonic movement that started ~25 Ma (million years ago) (Trewick *et al.*, 2007; Landis *et al.*, 2008; Neall & Trewick, 2008). Tectonic activity in the Miocene and Pliocene with substantial crustal uplift and orogenesis since the Pliocene resulted in the formation of the Southern Alps of the South Island (Kamp, 1992). This uplift produced considerable areas of mountainous and ecologically diverse habitat, especially in the South Island.

Notably however, one region of New Zealand has remained especially geologically active, because it is close to the point at which tectonic pressure at the contact of the Australian and Pacific continental plates transfers from strike-slip faulting to subduction (Trewick & Bland, in press). Reconstructions of paleogeographic distribution of land in central and lower North Island of New Zealand from the late Miocene times until today (ca. 6.5-0 million years (Myr)), shows that much of the landmass that currently comprises mid New Zealand (or southern North Island) is in geological terms very young, having only developed in the last 2-3 Myr (e.g. Beu, 1995; Bland *et al.*, 2008; Vonk & Kamp, 2008). Most parts of the southern North Island have only emerged above sea level in relatively recent times (ca. 1-2 Myr; Bland *et al.*, 2008), being submarine basins prior to this time (figure 3b.1) (e.g. Fleming, 1953; Kamp *et al.*, 2004). Cook Strait, separating the current North and South Islands of New Zealand, only formed in the mid Pleistocene (< 0.5 Myr). Since then, land connection between North and South Island only occurred when sea-levels lowered during glacial cycles (e.g. Nelson *et al.*, 2003). In this, in geological terms, young environment the expected species assemblage would express relatively low taxonomic and genetic diversity in lineages as well as species expansion from northern areas into southern North Island, as shown in other studies of plants and animals (see Wallis & Trewick, 2009).

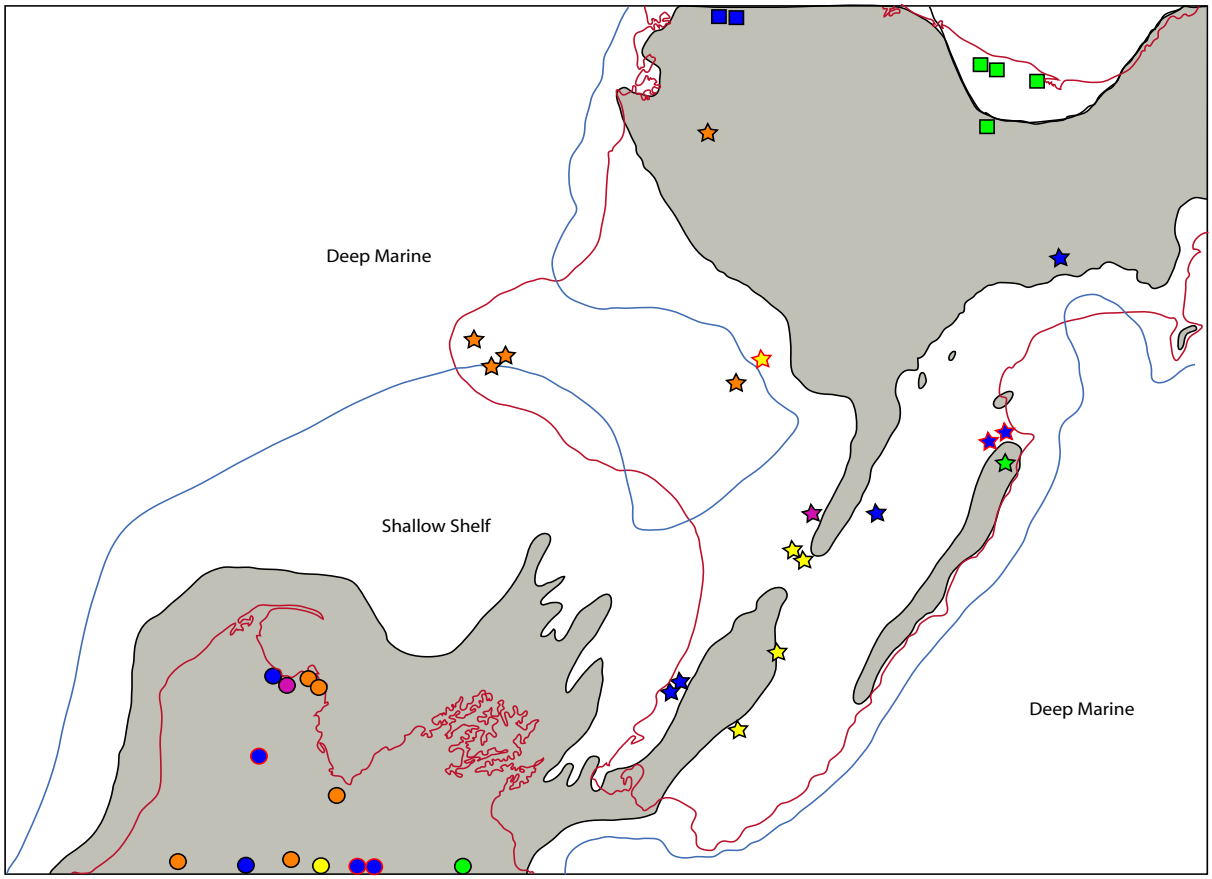

Fig. 3b.1: Reconstruction of the paleogeographic environment in New Zealand ca. 3Ma (Pliocene); grey areas outline land above sea level during this time (modified from Trewick & Bland); blue lines indicate border of deep marine to shallow shelf area around the coastline at this time; red lines depict the outline of present New Zealand land area. Symbols correspond to symbols in figure 3b.2 showing the present day sampling locations of sister taxa in North Island / South Island splits.

The beetle genus *Mecodema* is part of the Broscini tribe (Carabidae) that has a worldwide distribution but has its greatest diversity in the southern hemisphere (subfamily Nothobroscinae) (Roig-Juňent, 2000). The tribe Broscini consists of at least 27 genera, and the five genera present in New Zealand are all endemic and comprise 68 species (Britton, 1949). *Mecodema* is the biggest genus within New Zealand Broscini with 58 described species (Larochelle & Larivière, 2001). Adult beetles are relatively slow-moving, nocturnal, flightless, and usually scarce (Hutchison, 2001). The genus *Mecodema* is distributed throughout mainland New Zealand from alpine to coastal habitats and as far as the Chatham Islands, but most of the known species are geographically very restricted and not widespread.

In a previous study (chapter IIIa) the timing of radiation of *Mecodema* was inferred based on three mitochondrial DNA fragments (COI, COII and 16S) and a stratigraphic calibration point (emergence of the Chatham Islands). From this, unique substitution rates were calculated for the genes studied. In the present work the phylogenetic relationships and timing of the *Mecodema* radiation were examined further, and the result were considered in relation to up-to-date information on the formation of landscapes and other geophysical events in mid-New Zealand. In particular it was examined how well the lineage origination time obtained using a *Mecodema* gene-specific substitution rate (chapter IIIa) for the mitochondrial DNA COI fragment, correlates with estimates of the availability of land area in southern North Island.

Table 3b.1: Table of specimens used in this study. (a) List of specimens already used in chapter IIIa; (b) List of added samples; letters in the "genes" column refer to gene sequenced for each sample: a = COI; b = COII; c = 16S; d = 18S. Sample # refers to collection IDs, were applicable: L = Lincoln University Entomological Research Museum (LUNZ), P = S.M. Pawson collection;.(c) List of additional sequences used in this study taken from GenBank.

(a)

Sample #	genes	Sample ID	Species	Location
1a	a,b,c,d	MB 01	*M. alternans*	Chatham Is., South East Is.
1b	a,b,c	MB 02	*M. alternans*	Chatham Is., South East Is.
1c	a,b,c,d	MB 70	*M. alternans*	Chatham Is., Mangere Is.
1d	a,b,c	MB 71	*M. alternans*	Chatham Is., South East Is.
1e	a,b,c	MB 86	*M. alternans*	Chatham Is., Mangere Is.
1f	a,b,c	MB 87	*M. alternans*	Chatham Is., South East Is.
1g	a,b,c,d	MB 14	*M. alternans*	S.I., Dunedin, Taieri Mouth
1h	a,b,c,d	MB 16	*M. alternans*	S.I., Dunedin, Sandfly Bay
2a	a,b,c	MB 79	*M. crenicolle/crenaticolle*	S.I.,Marlborough Sounds, Pelorus Bridge, Circle Tr.
2b	a,b,c	MB 103	*M. crenicolle/crenaticolle*	S.I., Nelson Lakes, St. Arnaud Range, Wairau River
3	a,b,c,d	MB 66	*M. curvidens*	N.I., BOP, Rotorua
4	a,b,c,d	MB 110	*M. fulgidum*	S.I., Hanmer Springs, Clarence Valley, Mt. Percival
5	a,b,c	MB 91	*M.* cf *fulgidum*	S.I., Seaward Kaikoura Range, Mt. Lyford
6a	a,b,c,d	MB 98	*M. howittii*	S.I., Canterbury, Banks Peninsula, Otepatotu
6b	a,b,c	MB 99	*M. howittii*	S.I., Canterbury, Banks Peninsula, Otepatotu
7	a,b,c,d	MB 63	*M. longicolle*	N.I., Ruahine Ra., Pohangina Valley, Takapari Rd
8	a,b,c,d	MB 19	*M. lucidum*	S.I., Otago, Carrick Range
9	a,b,c,d	MB 11	*M.* nsp.	S.I., Central Otago, Obelisk top, Old Man Range
10	a,b,c	MB 37	*M.* nsp.	S.I., Arthurs Pass NP, Dome
11	a,b,c,d	MB 51.1	*M.* nsp.	N.I., Hawkes Bay, Havelock North
12	a,b,c,d	MB 68	*M. occiputale*	N.I., BOP, Mangatoi, Otanewainuku Forest
13	a,b,c,d	MB 21	*M.* cf *oconnori*	N.I., Wellington, Levin, Ohou, Browns Bush
14	a, b,c,d	MB 90	*M. oregoides*	S.I., Akuriri Scenic Res., Port Hills
15	a,b,c,d	MB 03	*M. rugiceps*	S.I., Fiordland, Lake Harris, Routeborn
16a	a,b,c,d	MB 45	*M. sculpturatum*	S.I., Dunedin, Ross Reserve
16b	a,b,c	MB 108	*M.* cf *huttense*	S.I., Mid Canterbury, Christchurch, Peel Forest
17a	a,b,c	MB 25	*M. simplex*	N.I., Manawatu, Palmerston North, Pahiatua Track
17b	a,b,c,d	MB 64	*M. simplex*	N.I., Manawatu, Palmerston North
18	a,b,c	MB 50	*M. spinifer*	N.I., Hawkes Bay, Mohi Bush
19	a,b,c,d	MB 18	*M. spiniferum*	N.I., Auckland, Waitakeres, Arataki
20	a,b,c,d	MB 96	*M. strictum*	S.I., Nelson, Takaka Hill, Canaan

21	a,,b,c,d	MB 95	*M. sulcatum*	S.I., Kaikoura, Reserve North of Ohau Point
22	a,b,c,d	MB 69	*M. validum*	N.I., Taupo, Tongariro NP, Whakapapanui Tr.k
	a,b,c,d	MB 106	*Meta. moniliferum*	S.I., Mid Canterbury, Christchurch, Quail Island
	a,b,c	MB 107	*Meta. moniliferum*	S.I., Mid Canterbury, Christchurch, Quail Island
	a,b,c	MB 13	*O.* nsp	S.I., Otago, Danseys Pass
	a,b,c,d	MB 41	*O. aereus*	S.I., Dunedin, Morrison St.
	a,b,c	MB 48	*D. clivinoides*	S.I., Seaward Kaikoura Range, Upper Tinline Va.
	a,b,c,d	MB 31	*D. clivinoides*	S.I., NW Nelson, Heaphy Track

(b)

Voucher	genes	Sample ID	Species	Location
	a,b	MB 86.1	*M. alternans*	Chatham Is., Mangere Is
	a	MB 88	*M. alternans*	Chatham Is., South East Is.
	a	MB 88.1	*M. alternans*	Chatham Is., South East Is.
	a,b	MB 190	*M. alternans*	S.I. Otago, Takahopa River Mouth
L2804	a	MB185	*M. alternans hudsoni*	The Snares
P15	a	MB 176	*M. rectolineatum*	S.I., Remarkebales Range south of Wye Creek
L2640	a	MB 196	*M. politanum*	S.I., Central Otago, Remarkables, Rastus Burn
	a,b	MB 128	*M. impressum*	S.I., Otago, Queenstown, Kinloch
	a,b	MB 125	*M. sculpturatum*	S.I., Southland, Catlins Forest Park, River Walk
	a	MB 111	*M. lucidum*	S.I.,Pisa Range, top hut 1680m
	a	MB 123	*M. fulgidum*	S.I., Hamner Springs, Forest Park, Fir Trail
	a	MB 134	*M. constrictum*	S.I., Canterbury, Craigeburn Park, Nature Walk
	a,b,d	MB 100	*M. costellum lewisi*	S.I., Road to Mt. White Station
	a,b	MB 101	*M. costellum obesum*	S.I.,Nelson,Takaka Hill,Parkland, Canaan
	a,b,d	MB 124	*M.* nsp.	S.I., Lewis Pass, Lewis Tops, ~ 1000m
L2372	a	MB 195	*M. allani*	S.I., Nelson Lakes,Matakitaki V.
L2383	a	MB 197	*M. laterale*	S. I., Fiordland, Routeburn Track
P23	a,b	MB 178	*M. minax*	S.I., Catlins, Mt. Pye Summit
P22	a,d	MB 180	*M. minax*	S.I., Catlins, Mt. Pye Summit
	a,b,d	MB 160	*M. elongatum*	S.I., Otago, Kinloch
	a,b,d	MB 143	*M. metallicum*	S.I., Buller, Fox River
	a,b	MB 117	*M. crenicolle/crenaticolle*	S.I., Nelson Lakes, Lake Rotoroa, Braeburn Walk
	a,b,d	MB 163	*M. crenicolle/crenaticollee*	N.I., Egmont NP, Kaiteke Ra., Lucy‘s Gully
	a	MB 186	*M..crenicolle/crenaticolle*	N.I., Waikato, Skyline Cave
	a,b,d	MB 121	*M. ducale*	S.I., Lewis Pass, Lake Daniels Walk
	a,b,d	MB 147.1	*M. oregoides*	S.I., MC, Ahuriri Scenic Reserve

	a	MB 30	*M.* undet.	S.I., Able Tasman, Awaroa
	a	MB 38	*M.* undet.	S.I., Takaka, The Grove
	a	MB 49	*M.* undet.	S.I.,Takaka, Rameha Track
	a,b	MB 65	*M. occiputale*	N.I., BOP, Ohope Scenice Res.,Fairbrother Walk
	a	MB 67	*M. occiputale*	N.I., BOP, Mangatoi,Otanewainuku Forest,Rimu Tr.
	a	MB 61	*M. crenicolle/crenaticolle*	N.I., Taranaki, Eltham, Lake Rotokare
	a,d	MB 62	*M. crenicolle/crenaticolle*	N.I., Taranaki, Eltham, Lake Rotokare
	a	MB 72	*M. crenicolle/crenaticolle*	N.I., Ohakune, Visitor Centre, Mangawhero Forest Tr.
	a	MB 12	*M. crenicolle/crenaticolle*	S.I., Nelson, Shenandoah
	a	MB 44	*M. crenicolle/crenaticollee*	S.I.,Able Tasman, Awaroa,Dacha
	a	MB 82	*M. crenicolle/crenaticolle*	S.I.,Able Tasman, Awaroa,Dacha
	a	MB 54	*M. morio*	S.I.,Purakaunui Falls, Catlins
	a,b,d	MB 105	*M. infimate*	S.I., Codfish Island
	a,b	MB 76	*M. simplex*	N.I., Wairarapa,Tararua Forest Park, Putara
	a,d	MB 77	*M. simplex*	N.I., Tararua Ra., Mt Holdsworth, Powell Hut
	a,d	MB 35	*M. constrictum*	S.I., Fog Peak, Porter‘s Pass
	a,b	MB 50.1	*M. spinifer*	N.I.,Hawkes Bay, Mohi Bush
	a,b	MB 81	*M. fulgidum*	S.I.,Kahurangi NP, Cobb Valley, Lake Sylvester Tr.
	a,b	MB 27	*M. constrictum*	S.I., Canterbury, Craigeburn Rec. area
	a	MB 07	*M.* nsp.	S.I., Seaward Kaikoura Ra., Fyffe Palmer Scenic Res.
	a,b	MB 20	*M.* cf *oconnori*	N.I., Levin, 30B The Avenue
	a,d	MB 73	*M.* cf *oconnori*	N.I., Te Urewera,L. Waikaremoana,Ngamoko Trig Tr.
	a	MB75	*M.* cf o*connori*	N.I., Wairarapa, Dannevirke, Norsewood
	a	MB 10	*M. spiniferum*	N.I., Auckland, Waitakares, Opanuku Rd.
	a	MB 17	*M. punctatum*	S.I., Rock+Pillar Range
	a	MB 04	*M. sculpturatum*	S.I.,Dunedin, Leith Saddle, Swampy Summit
	a	MB 06	*M. sculpturatum*	S.I., Dunedin, Mosgeil, Silver St
	a,d	MB 09	*M. huttense*	S.I., Canterbury, Peel Forest
	a,b	MB 46	*M.* cf *huttemse*	S.I., Canterbury, Peel Forest
L3089	a	MB 198	*Meta. aberrans*	S.I., Canterbury, Mackenzie, Lake Tekapo
	a	MB 187	*Meta. aberrans*	S.I., Otago, Cromwell, Bendigo
P16	a	MB 181	*Meta. aberrans*	S.I., Otago, Old Man Range, Omeo Gully
P17	a	MB 182	*Meta. aberrans*	S.I., Otago, Old Man Range, Omeo Gully
P10	a	MB 184	*Meta. aberrans*	S.I., Otago, Old Man Range, Omeo Gully
L3088	a	MB 194	*Meta. tibiale*	S.I., Central Otago, Upper Clutha
	a,b,d	MB 85	*Brullea antarctica*	N.I.,Manawatu,Himatangi Beach
	a	MB 199	*Bountya insularis*	Bounty Is., Proclamation Is.
(3574)	a	MB 192	*Chylnus ater*	Australia

a,b	MB 161	*D. obtusum*	S.I., Fiordland, Kepler Track
a,b,d	MB 162	*D. obtusum*	S.I., Otago, Catlins Coast, Tautuku
a,b,d	MB 159	*D. clivinoides*	S.I., Otago, Kinloch
a	MB 8	*D. clivinoides*	S.I., NW Nelson, Cobb Valley
a	MB 175	*D. seclusum*	S.I., Fiordland, Spey River Valley
a,b	MB 28	*O. aereus*	S.I., Dunedin, 46 Morrison St.
a	MB 29	*O. aereus*	S.I., Dunedin, Sandfly Bay
a,b	MB 47	*O. aereus*	S.I., S.I.,Dunedin, Silver Peaks
a	MB53	*O. aereus*	S.I., L. Onslow, Lammarlaws
a,b	MB 5	*O. inaequalis*	S.I., Dunedin, Miller Rd., Waitati

(c)

Species	gene	GenBank accession	Reference
Oregus septentrionalis	COI	AF466847	Pawson *et al.*, 2003
Oregus septentrionalis	COI	AF466848	Pawson *et al.*, 2003
Oregus crypticus	COI	AF543423	Pawson *et al.*, 2003
Oregus inaequalis	COI	AF466850	Pawson *et al.*, 2003
Calathus aztec	COI	GU254333	Ruiz *et al.*, 2010

IIIb.2 MATERIAL & METHODS

Sampling

Sampling for this study supplements existing collection of specimens of the genus *Mecodema* (Carabidae: Broscini). In addition to the samples already used for analyses in chapter IIIa a further 85 specimens were sequenced (Table 3b.1a&b). As many of the species in this genus are scarce and therefore difficult to collect we also made use of material from museum collections. In total the sampling comprised 118 specimens, with 88 specimens of *Mecodema* representing 37 described species, and 4 undescribed species (I. Townsend, pers. comm.). In addition, an outgroup comprised other New Zealand Broscini; *Oregus* (Putzeys, 1868) (7 specimens), *Diglymma* (Sharp, 1886) (7), *Brullea antarctica* (Laporte de Castelnau, 1867) (1), *Metaglymma* (Bates, 1867) (8), and *Bountya insularis* (Townsend, 1971) (1); one beetle of the Australian genus (*Chylnus ater* (Putzeys, 1868), and a carabid outside the Broscini tribe *Calathus aztec* (Ball & Negre, 1972). Outgroup sampling was supplemented by GenBank sequences (Table3b.1c). Fresh specimens were preserved in 95% ethanol after hand collection in the field, and stored with unique voucher numbers at Massey University, Palmerston North. Specimens

Table 3b.2: Table of primers designed for and used in this study, incorporating the IUPAC code. Primers denoted with * were taken from Simon *et al.*, 1994.

Primer name	Sequence	Gene
MB_138R	3' GCTGATGTAAAATATGCTCG	COI
MB_139F	5' GAGCATATTTTACATCAGCAAC	COI
MB_403R	3' CAATGAATAAATCCTCCAA	COI
MB_405F	5' GGAGGATTTATTCATTGAT	COI
MB_591R	3' GTWGATCCAATTGATGAAAC	COI
MB_588F	5' GTARTTTCATCAATTGGATC	COI
MB_812R	3' CTTAAATATGATCATGTRG	COI
mtD10*	5' TTGATTTTTTGGTCATCCAGAAGT	COI
mtD12*	5' TCCAATGCACTAATCTGCCATATTA	COI
mtD13*	5' AATATGGCAGATTAGTGCA	COII
mtD18*	5' CCACAAATTTCTGAACATTGACCA	COII
mtD20*	5' GTTTAAGAGACCAGTACTTG	COII
mtD29*	5' GGTCCCTTACGAATTTGAATATATCCT	16S
mtD34*	5' CGCCTGTTTAACAAAAACAT	16S
nuc42*	5' TAATGATCCTTCCGCAGGTTCA	18S
nuc43*	5' TCCCTGGTTGATCCTGCCAGTA	18S

from other museum collections used in this study are labelled with the appropriate collection specific voucher numbers (Table 3b.1a & b). Species identification relied on morphological characters (Britton, 1949).

DNA extraction, amplification and sequencing

DNA was extracted from a single leg of each specimen using either a salting-out extraction protocol (Sunnucks & Hales, 1996) or, for previously pinned and dried samples, a CTAB and phenol/chloroform extraction (Trewick, 2008). Partial cytochrome oxidase I (COI) (788 bp) was amplified using primers C1-J-2195 and L2-N-3014 (Simon *et al.*, 1994). For some of the pinned museum specimens DNA extractions were undertaken in a dedicated ancient DNA laboratory, remote from modern DNA facilities, using a CTAB and phenol/chloroform extraction

(Trewick, 2008), following stringent protocols for handling ancient DNA (Willerslev & Cooper, 2005). For these specimens, which were expected to yield fragmented, low concentration DNA. *Mecodema*-specific COI primers using the program Oligo4 (Molecular Biology Insights, Inc., Cascade, CO) were designed in order to generate a series of short (~130 – 200 bp) overlapping fragments (Table 3b.2). For a subset of 72 specimens a 590bp fragment of partial cytochrome oxidase II (COII) was sequenced using the primers TL2-J-3034 and C2-N3661 or TK-N3785 (Simon *et al.*, 1994). For an additional subset of 42 Broscini a 970 bp fragment of nuclear rRNA (18S) was also sequenced using primers 18S-S22 and 18S-A1984 (Vawter, 1991).

Polymerase chain reactions were performed in 10 µl volumes. The amplified products were checked on a 1% agarose gel and purified using SAP/EXO1 digest (USB Corporation) following the manufacturer's instructions. Purified PCR products were sequenced using standard protocols for the ABI Prism BigDye Terminator Ready Reaction Kit (Applied Biosystems, Mulgrave, Australia) and run on an ABI Prism 377 automated sequencer (Applied Biosystems). Sequence identity was confirmed by comparison with published data and checked for nucleotide ambiguities in Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI, www.genecodes.com) and aligned using Se-Al v2.0a11 (Rambaut, 1996).

Phylogenetic analysis

Phylogenetic trees were rooted using the outgroup taxa listed above, either with only New Zealand Broscini (COII and 18S) or including the other three species outside of New Zealand Broscini (COI). Neighbor-joining (NJ) analysis, as implemented in PAUP* with the COI, COII and 18S data, and Bayesian analyses using Mr.Bayes 3.1.2 (Ronquist & Huelsenbeck, 2003) were conducted. Analysis of each gene data set used a sample specific six parameter model, GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites, selected by jModelTest 3.5 (Guindon & Gascuel, 2003; Posada, 2008). Four independent MCMC runs for ten million generations with a burn in of 10% were employed. Resulting posterior probabilities on the nodes were recorded.

Molecular dating

For molecular dating of the COI dataset we used the software BEAST v1.5.4 (Drummond & Rambaut, 2007). All analyses were conducted with a Yule tree prior and a randomly generated starting tree under the GTR+I+Γ model of nucleotide substitution. We used the substitution rate of 1.18%/Myr as a prior for the analyses. This rate was the one calculated in the previous chapter (chapter IIIa) for *Mecodema* COI. The MCMC was run for 40 million generations, sampling every 1000th step after a discarded burn-in of 1000 sampled trees. The analysis was run four times to test for convergences of the output. The program Tracer v1.5 (Drummond & Rambaut, 2007) was used to summarize posterior distributions of all parameters, to verify convergence of the MCMC and to estimate Effective Sample Sizes (ESS). Posterior distributions of all parameters were estimated from the combined posterior distributions of all runs. The program FigTree v1.3.1 (Drummond & Rambaut, 2007) was used to visualize the reconstructed phylogenies.

IIIb.3 RESULTS

The complete alignment of the COI gene region comprising 118 specimens (including 26 outgroup specimens within Broscini and another three outside of the tribe) was 788 bp long and had an overall A-T bias of 73%.

Diglymma and *Oregus* species, that represent two of the New Zealand Nothobroscina genera taxonomically closest to *Mecodema* (Roig-Juňent, 2000) grouped, as expected, outside of the *Mecodema* clade (figure 3b.2). The other putative sister taxa to *Mecodema* within Broscini, *Metaglymma* and *Brullea,* were not supported as being sister to *Mecodema*, but were nesting within the *Mecodema* radiation. The relationship among *Mecodema* species was corroborated by the present dataset, with groupings similar to proposed taxonomic clades by Larochelle & Larivière (2001) and as already discussed in chapter IIIa (figure 3b.2). These groupings were also apparent in the Bayesian calculation of the 18S (figure3b.3) and COII (result not shown) datasets, although the overall resolution of the tree was not sufficient to infer sister taxa relationships within or between the groups as most of the *Mecodema* radiation is very shallow, with estimates of many clade origins less than 5 Myr.

There is little spatial correlation within the COI hypothesis, instead one observes several North Island/South Island (NI/SI) splits within the phylogram (figure 3b.2), at least one in each of the taxonomic groups in *Mecodema*. All but one of these splits (the split between the simplex/validum group to the rest of the inner clade) is younger than 5 Myr. All of the splits between North and South Island sister taxa include specimens now distributed in the mid or lower North Island (figure 3b.1) in areas that have been below sea level in recent geological times.

Brullea antarctica is a special case as this monotypic genus can (theoretically) be found on beaches in both North and South Island, but for this study only one specimen from lower North Island was available, that no inference could be drawn from this taxon.

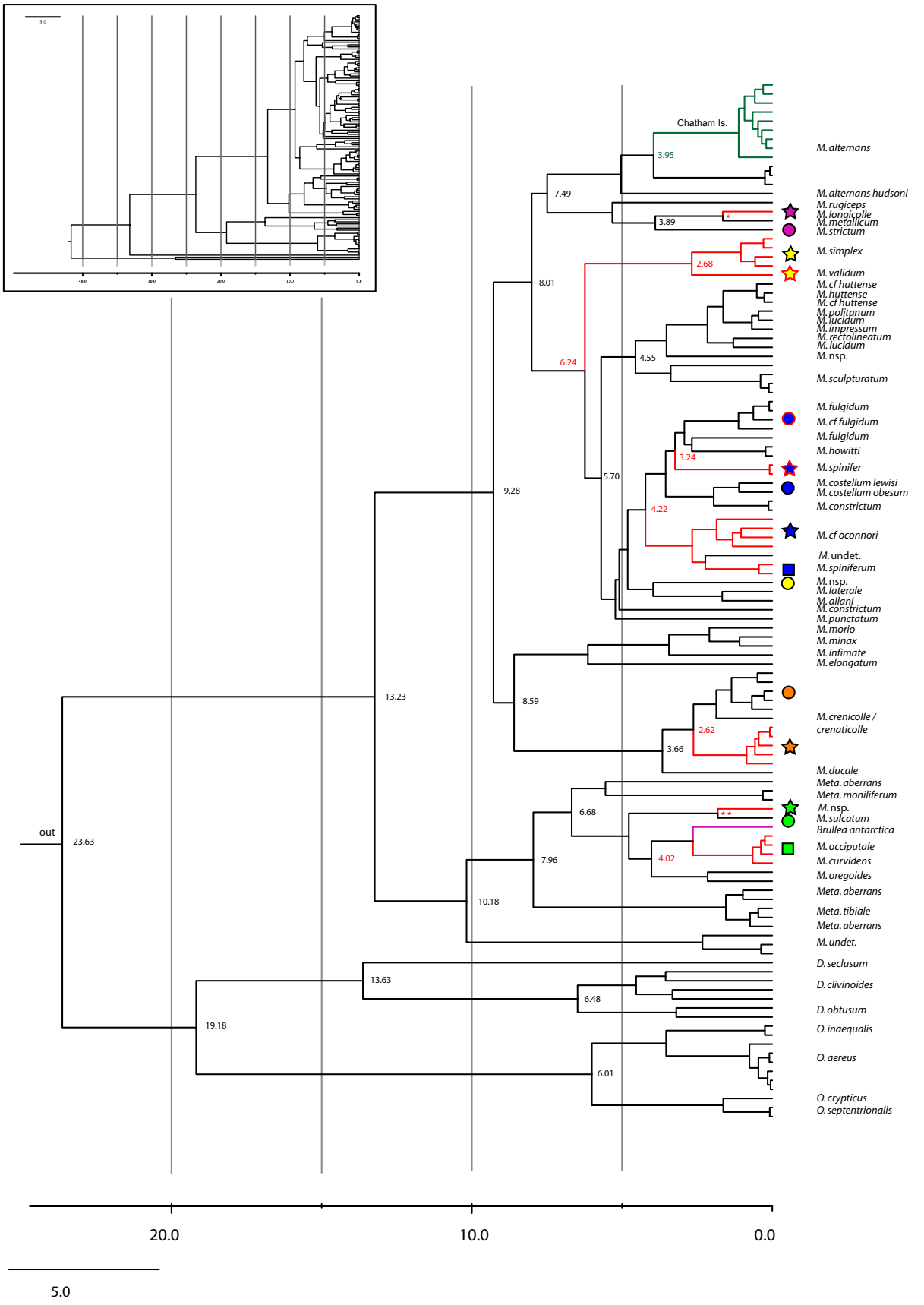

Fig. 3b.2: COI Bayesian tree generated in BEAST with numbers on nodes giving age estimates calculated with the fixed substitution rate of 0.0059 (* = 1.81 Myr; ** = 1.65 Myr), outgroups have been cut. Red branches indicate lineages present in North Island New Zealand, branches of South Island lineages are coloured in black and the Chatham Islands lineage is coloured in green. Symbols on tips correspond to symbols in figure 3b.1, showing disjunct distributions of taxa in lower North Island and northern South Island of New Zealand.Inset: showing the same COI tree, but with outgroup taxa (*Chylnus ater*, *Bountya insularis* and *Calathus aztec*) included.

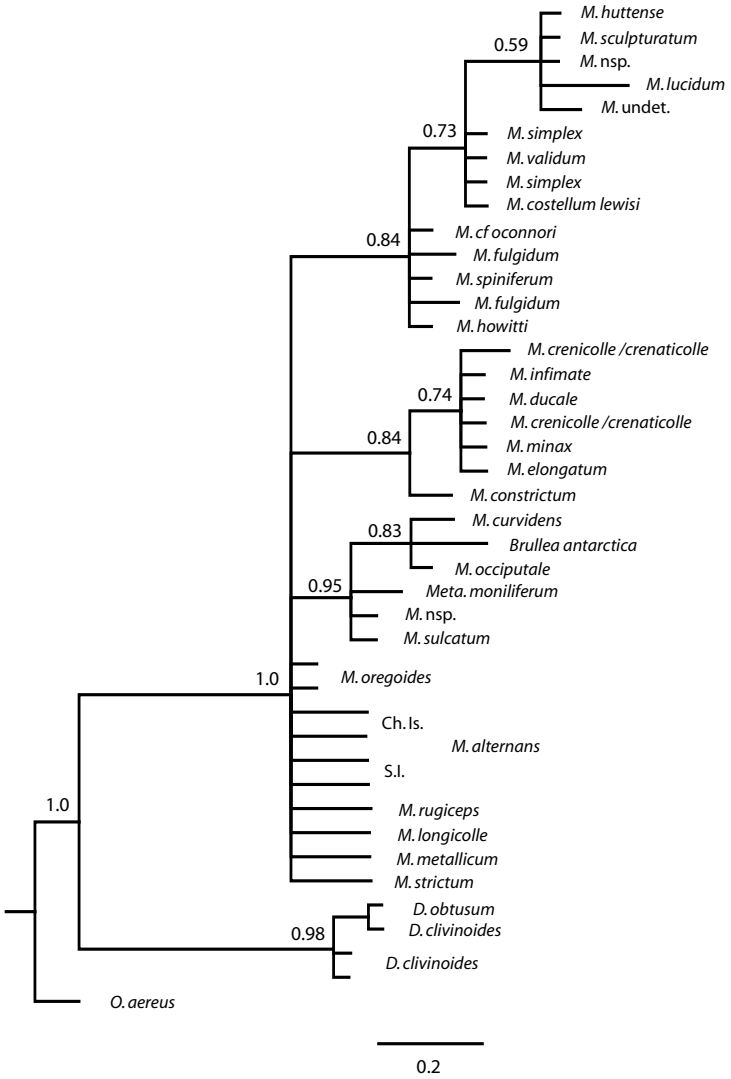

Fig. 3b.3: Bayesian tree of the 18S fragment of a subset of Broscini species from New Zealand. Numbers above branches show Bayesian posterior probabilities.

IIIb.4 DISCUSSION

In a previous study (chapter IIIa), species radiation of *Mecodema* beetles was analysed and molecular dating undertaken using stratigraphic information about the emergence of land in the vicinity of the Chatham Islands. As the Chatham Islands are occupied by a species of *Mecodema*, and this population could not have existed prior to the availability of land, the maximum age of the inferred New Zealand-Chatham phylogenetic split was estimated by the maximum age of the Chatham Islands. This yielded an estimate for the rate of molecular evolution for several genes in *Mecodema*. As previously noted (chapter IIIa), these rates could be underestimates if (all else being equal), *Mecodema* did not arrive soon after the Chatham Islands formed, but in fact more recently. Thus, the estimated rate of molecular evolution is likely to be conservative,

and comparison with other published rates supports this inference. In this study the timing of speciation within the genus *Mecodema* was investigated further in correlation with land formation in New Zealand, and in particular the fit of date estimates with the availability of habitat / land in the most geologically youthful region of southern North Island was examined.

The phylogenetic pattern within *Mecodema* is generally more consistent with the taxonomic subgroups proposed by morphology (Larochelle & Larivière, 2001), than with geographic regions. In the COI, COII and 18S data, there is, for instance, no support for North Island / South Island monophyly, even though a simple prediction of allopatric speciation is that such a correlation might exist. Among clades there is no strong signal of regional partitioning; in many instances clades and subclades contain both North Island and South Island species although it is notable that very few species are found in both islands.

On the basis of a conservative rate estimate for molecular evolution most diversification seems to have happened since the Pliocene (> 5 Myr). The inference from this is that speciation and extinction within this genus has been strongly influenced by relatively recent paleogeographic processes, such as mountain building in the Pliocene and climate cycling in the Pleistocene. Given the relationships within *Mecodema* (figure 3b.2) and the calibration used, at least eight exchanges between North Island and South Island of New Zealand can be inferred. Considering the availability of land during this time frame, the implication is that these exchanges happened from south to north. South Island has been of similar size as it is today, at least since the Pliocene, whereas southern North Island formed only in the last 1 or 2 million years. Prior to this, the size of the proto-North Island was considerably less than today, and its southern coast was geographically further from northern South Island than it is today. The splits between sister taxa currently occupying either North or South Island range in estimated age from 6.24 Myr to 1.65 Myr. This suggests numerous exchanges between these regions since the Pliocene, involving most probably colonisation after over-sea dispersal, rather than in situ radiation from a single ancestor in each island. The dominance of South Island lineages within *Mecodema*, paraphyletic (e.g. *Metaglyma*) and sister clade (*Diglyma, Oregus*) are consistent with the notion that colonisation has for the most part been northwards.

The current sampling is not complete in terms of species or populations, and additional sampling is most likely to reduce the inferred age of formation of lineages, and might increase the number of inferred dispersal events. However, a number of observations can be made: Geographically proximate species are rarely sister taxa (e.g. *M. costellum* blue spot, *M. crenicolle* orange spot, *M. strictum* pink spot in north east South Island; figures 3b.1 & 2). Localised species might have arisen via allopatry on past islands (e.g. *M.* n.sp – green star, in eastern central North Island). Many populations and species have their range (sampled and known) in areas that were under water during late Pliocene and early Pleistocene (e.g. *M. crenicolle/ crenaticolle* orange stars, *M. oconnori* blue stars, *M. simplex* yellow stars). The existence of these latter taxa indicates speciation since land became available and we note a mismatch between estimated

lineage age and estimate of land age. For example the split between North Island and South Island *M. crenicolle/ cranaticolle* complex beetles is estimated at 2.68 Ma. This predates any evidence for land in southern North Island by more than 1 Myr, and predates by at least 1.5 Myr the most substantial period of land connection between North Island and South Island in the late Pleistocene. One explanation for this type of mismatch between dates derived from genetics and those from geology is that lineages evolved before the extant species they yielded arrived where they occur today, and it is well recognised that species ranges do change over time. In North Island New Zealand, molecular studies showing this type of range expansion include tree weta (Morgan-Richards *et al.*, 2001). However, evidence of recent range expansion is most easily found when populations of the taxon concerned still exist at source locations as well as colonised locations. In *Mecodema*, range shift after lineage orgination would require lineage splitting, persistence of two lineages at a source location, expansion of at least one lineage and extinction of that lineage at the source. An alternative and simpler explanation is that the molecular rate applied to these data is an underestimate, which has thus yielded over estimation of lineage age.

Thus, as identified previously (chapter IIIa), even the most stringent algorithms and best available calibration points cannot be expected to always yield an accurate evolutionary history. Comparison of inferred node ages with additional evidence in this case implies that the *Mecodema* COI evolutionary rate is indeed conservative and provides only an upper bound for ages of speciation in this genus. This suggests that diversification in *Mecodema* may well be even younger than already inferred and making this an exciting example of a very recent impressive invertebrate radiation in New Zealand.

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IV

Population structure and biogeography of *Hemiphaga* pigeons (Aves: Columbidae) on islands in the New Zealand region*

ABSTRACT

The New Zealand avifauna includes lineages that lack close relatives elsewhere and have low diversity, characteristics sometimes ascribed to long geographic isolation. However, extinction at the population and species level could yield the same pattern. A prominent example is the ecologically important pigeon genus *Hemiphaga*. In this study we examined the population structure and phylogeography of *Hemiphaga* across islands in the region. Mitochondrial DNA was sequenced for all species of the genus *Hemiphaga*. Sixty-seven individuals from mainland New Zealand (*Hemiphaga novaeseelandiae novaeseelandiae*), six of the Chatham Islands sister species (*Hemiphaga chathamensis*) and three of the extinct Norfolk Island subspecies (*Hemiphaga novaeseelandiae spadicea*) were included in this study. Novel D-loop and cytochrome *b* primers were designed to amplify DNA from museum samples. Additionally five other mitochondrial genes were used to examine placement of the phylogenetic root. Analyses of mitochondrial DNA sequences revealed three *Hemiphaga* clades, consistent with the allopatric populations of recognized (sub)species on oceanic islands. Of the 23 D-loop haplotypes among 67 New Zealand pigeons (*Hemiphaga n. novaeseelandiae*), 19 haplotypes were singletons and one haplotype was common and widespread. Population genetic diversity was shallow within and between New Zealand populations, indicating range expansion with high interpopulation exchange. Tentative rooting of the *Hemiphaga* clade with cyt *b* data indicates exchange between mainland New Zealand and the Chatham Islands prior to colonization of Norfolk Island. We found low genetic divergence between populations on New Zealand, the Chatham Islands and Norfolk Island, but deep phylogenetic divergence from the closest living relatives of *Hemiphaga*. The data are consistent with the hypothesis of population reduction during the Pleistocene and subsequent expansion from forest refugia. Observed mobility of *Hemiphaga* when feeding helps explain the shallow diversity among populations on islands separated by many hundreds of kilometres of ocean. Together with comparison of distribution patterns observed among birds of the New Zealand region, these data suggest that endemicity might not represent long occupancy of an area, but descent from geologically recent colonizations. We consider the role of lineage pruning in creating the impression of old endemicity.

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IV.1 INTRODUCTION

One of the reasons New Zealand intrigues evolutionary biologists is that it displays biological characteristics of both oceanic islands and continental land masses (Daugherty *et al.*, 1993; Trewick & Morgan-Richards, 2009). It is continental in stratigraphic composition and origin (founded on a tectonic fragment, Zealandia, separated from Gondwanaland ~83 Ma) and is often viewed as continental in its biology (Cowie & Holland, 2006). However it is increasingly apparent that the New Zealand biota is a dynamic and relatively young evolutionary system (McGlone, 2005; Goldberg *et al.*, 2008; Wallis & Trewick, 2009). Following its separation from Gondwanaland starting 83 Ma, most if not all of the continent of Zealandia submerged beneath the sea (Pole, 1994; Waters & Craw, 2006; Trewick *et al.*, 2007; Landis *et al.*, 2008). The main islands of New Zealand, which are a small emergent part of Zealandia, are primarily the product of tectonic activity initiated ~25 Ma (Trewick *et al.*, 2007; Landis *et al.*, 2008; Neall & Trewick, 2008). Other emergent parts of Zealandia include New Caledonia, Norfolk Island and the Chatham Islands (figure 4.1), all of which appear to be the products of volcanic or tectonic activity. If any land persisted from Zealandia through to modern New Zealand, it was at its smallest towards the end of the Oligocene and is likely to have resulted in population bottlenecks and lineage extinction (Cooper & Cooper, 1995). Tectonic activity in the Miocene and Pliocene resulted in substantial remodelling of the archipelago and culminated in crustal uplift and orogenesis since the Pliocene (Kamp, 1992). Furthermore, substantial biotic turnover occurred during the early Miocene (as evident from the fossil record, Lee *et al.*, 2001) and Plio-Pleistocene time when the forests habitats repeatedly retreated and expanded during climate cycling (McGlone *et al.*, 2001). During glacial phases, forests appear to have been restricted to small areas mostly in northern North Island and north-western South Island of New Zealand, with the remaining vegetation being dominated by shrub and grassland (McGlone, 1985; Alloway *et al.,* 2007). How the biota of the New Zealand region has responded to the geophysical history is of particular interest to biologists attempting to reconcile the region's old continental geological history with the observation of a more island-like biotic composition (e.g. Falla, 1953).

Figure 4.1: The New Zealand region with an approximation of Zealandia indicated in grey.

In the face of such intense climatic and geological activity it is not surprising that phylogeographic studies of New Zealand's fauna have suggested that Pleistocene climate

cycling had an important influence on the distribution and divergence of several taxa (Buckley *et al.*, 2001; Trewick & Wallis, 2001; Neiman & Lively, 2004; Hill *et al.*, 2009; for a review see Wallis & Trewick, 2009) although other lineages appear to have retained diversity that dates to earlier climatic and geophysical changes of the late Pliocene (Trewick *et al*., 2000; Trewick & Wallis, 2001; Buckley & Simon, 2007). Among phylogeographic studies of New Zealand vertebrates, some reveal patterns consistent with habitat fragmentation during glacial periods of the Pleistocene (e.g. the short-tailed bat, *Mystacina tuberculata* – Lloyd, 2003; brown kiwi, *Apteryx* spp. – Baker *et al*., 1995), whereas recent speciation in Plio-Pleistocene time is evident in a number of New Zealand forest birds (e.g. kokako, *Callaeas cinerea* – Murphy *et al.*, 2006, parakeets – Boon *et al*., 2001*a*; kiwi – Baker *et al.*, 1995; Burbidge *et al.*, 2003, moa – Baker *et al.*, 2005; Bunce *et al.*, 2009, and robins, *Petroica* spp. – Miller & Lambert, 2006) (for further discussion see Goldberg *et al.*, 2008; Wallis & Trewick, 2009).

A key observation from the study of New Zealand birds is that the assemblage, while relatively small, is drawn from the range of global phylogenetic diversity, rather than being a monophyletic group (Trewick & Gibb, 2010). There is endemicity, which is suggestive of isolation, but this is at many taxonomic levels from family to subspecies, and so it could be inferred that different elements of the avifauna have experienced different degrees of isolation (Trewick & Gibb, 2010). The geographic ranges and taxonomic distinctiveness of forest birds in the wider New Zealand (Zealandian) region are similarly diverse (figure 4.2). For example, *Hemiphaga* pigeons occur on mainland New Zealand, the Chatham Islands and Norfolk Island (figure 4.2a); whereas *Cyanoramphus* parakeets occur on mainland New Zealand, the Chatham Islands, Norfolk Island and New Caledonia, which also harbours the sister genus (figure 4.2b); New Zealand has some endemic petroicid robins but the main diversity is in Australia with evidence for separate exchanges between Australia and other islands (figure 4.2c); and one species of *Ninox* owl is found in New Zealand and also on Norfolk Island, New Caledonia (exinct) and Australia, but most species richness is in Australia and Asia (figure 4.2d).

One inference from genera that are restricted to the New Zealand region, such as *Hemiphaga* pigeons, is that they have been isolated there for a comparatively long time, but an alternative explanation is that they arrived more recently and have since been extirpated elsewhere. For example, if *Cyanoramphus* was extinguished in New Caledonia, the genus would be rendered endemic to the New Zealand region (figure 4.2b). Such an inference might be considered less parsimonious because dispersal is often considered to be an unlikely phenomenon (see Cook & Crisp, 2005 for discussion). One way to explore the plausibility of this alternative is to examine the population genetic structure of New Zealand taxa to see if they have retained the capacity for recent exchange. If they do, we cannot exclude the possiblity that deeper level endemicity in New Zealand is a result of recent extinction elsewhere.

Therefore, in this study we examined the phylogeography of *Hemiphaga* pigeons, which are ecologically important forest birds and endemic to the New Zealand region. The genus *Hemiphaga* is considered to be part of the radiation of Southeast Asian pigeons and doves (Gibbs *et al.*, 2001; Johnson, 2004). It belongs to a small group of four species-poor genera

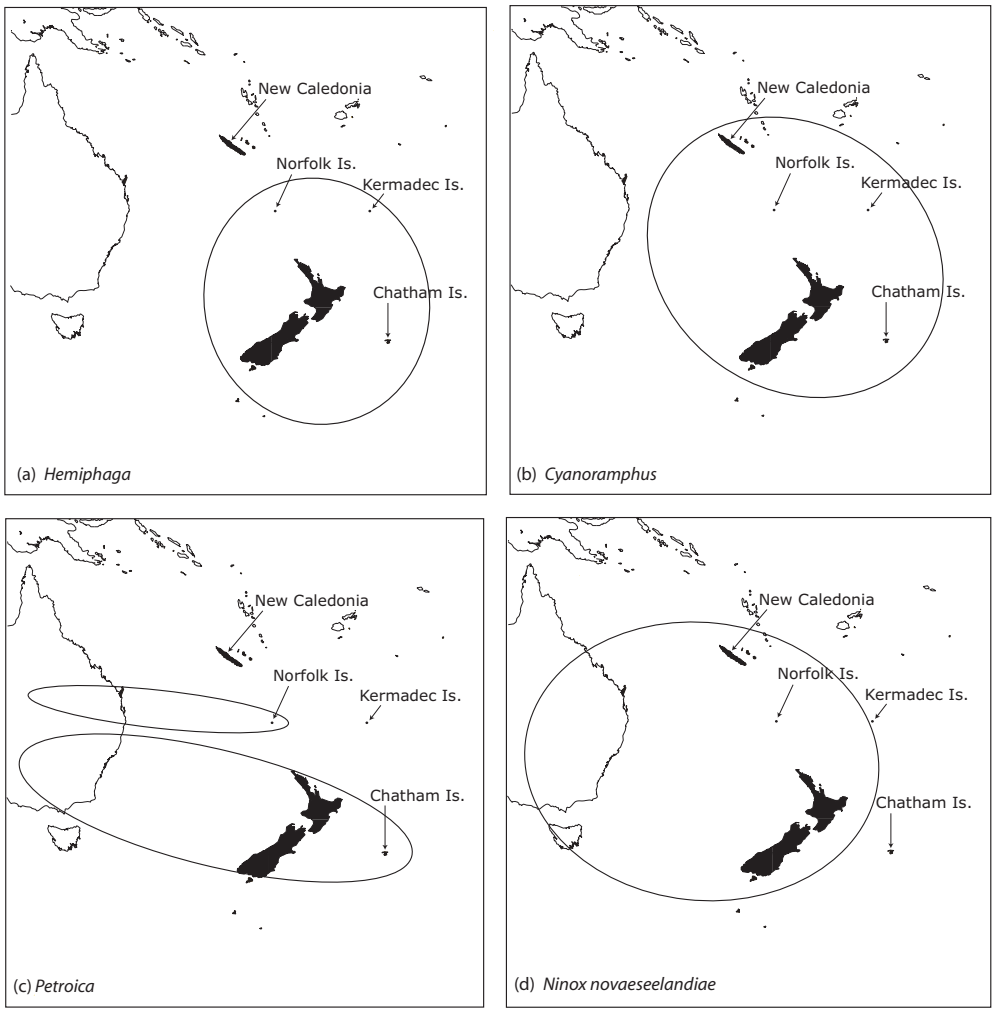


Figure 4.2: Four paradigms of forest bird distributions among islands focusing on the New Zealand region: (a) pigeon (*Hemiphaga* spp.), (b) parakeet (*Cyanoramphus* spp.), (c) robin (*Petroica* spp.), (d) owl (*Ninox novaeseelandiae*). Ellipses encompass land areas from where representatives of each taxon have been recorded either in the extant native avifauna or in Holocene fossils (Worthy & Holdaway, 2002; Clements, 2007). *Hemiphaga* (a) and *Cyanoramphus* (b) are endemic to islands in the areas indicated, whereas *Petroica* (c) has additional diversity in Australia, and *Ninox novaeseelandiae* (d) has most diversity in Australia and Asia.

(*Hemiphaga* (2 species), *Lopholaimus* (1 species), *Cryptophaps* (1 species) and *Gymnophaps* (3 species)) that might be remnants of an earlier higher species diversity (Gibbs *et al.*, 2001). Species of *Gymnophaps* occur on the Moluccas, New Guinea and the Solomon Islands, and *Cryptophaps poecilorrhoa* (Brüggemann, 1876) is endemic to Sulawesi (Gibbs *et al.*, 2001). *Lopholaimus antarcticus* (Shaw, 1794) is endemic to the east coast of Australia, and based on morphology it has been suggested that it is the closest living relative of *Hemiphaga* (Gibbs *et al.*, 2001). However, using molecular data Pereira *et al.* (2007) showed that *Lopholaimus* grouped with *Gymnophaps*, with *Hemiphaga* being sister to these, and new molecular results corroborate this (Gibb & Penny, 2010). In contrast, other genera in the Pacific fruit pigeon clade are both species-rich and widely distributed through northern Oceania (Gibbs *et al.*, 2001). The known distribution of the genus *Hemiphaga* includes mainland New Zealand, the Chatham Islands and formerly Norfolk Island and Kermadec Islands (Worthy & Brassey, 2000). Two

extant species of *Hemiphaga* are generally recognized. *Hemiphaga novaeseelandiae* (Gmelin, 1789) consists of two subspecies. *Hemiphaga novaeseelandiae novaeseelandiae* (Gmelin, 1789) is found throughout mainland New Zealand (locally called kereru) and formerly the Kermadec Islands, but is known from there by a single subfossil bone only (Worthy & Brassey, 2000). *Hemiphaga novaeseelandiae spadicea* (Latham, 1802) was described from Norfolk Island (figure 4.1), where it became extinct in the mid 1800s (Tennyson & Martinson, 2006). The sister species, *H. chathamensis* (Rothschild, 1891) (locally known as parea) occurs in the Chatham Islands and was recently attributed species status based on morphometric evidence (Millener & Powlesland, 2001).

The New Zealand woodpigeon (*Hemiphaga n. novaeseelandiae*) is a large (510–850 g) forest bird (Clout, 1990; Higgins & Davies, 1996), that feeds on buds, leaves, flowers and fruits from a wide variety of forest tree and shrub species (McEwen, 1978; Higgins & Davies, 1996). It is the only extant and widespread disperser of fruits with large seeds in the New Zealand islands (Clout & Hay, 1989; Lee *et al.*, 1991; Kelly *et al.*, 2006). Individual pigeons occupy small home ranges (~ 1 ha) for short periods when food is readily available (Clout & Hay, 1989), but also travel tens of kilometres between seasonal feeding sites (Clout *et al.*, 1986, 1991; Millener & Powlesland, 2001; Harper, 2003; Powlesland *et al.*, 2007). Little is known about the ecology and behaviour of the extinct Norfolk Island pigeon (*H. n. spadicea*), but it is assumed that it exhibited traits similar to the mainland New Zealand and Chatham Islands pigeons (Tennyson & Martinson, 2006). All three taxa were once numerous in forested areas (Atkinson & Millener, 1991; Worthy & Holdaway, 2002), but were reduced drastically in the 18th and 19th centuries by hunting, deforestation and introduced mammalian predators, which ultimately resulted in the extinction of the Norfolk Island subspecies (Tennyson & Martinson, 2006).

Subfossil bones and fossils of pigeons in the New Zealand region include a small ground dove (*Gallicolumba norfolciensis*) native to Norfolk Island, which became extinct in the late 1800s after human contact (Worthy & Holdaway, 2002; Tennyson & Martinson, 2006), and at least two taxa from mainland New Zealand in Miocene time (Worthy *et al.*, 2009), but there are no other taxa in the recent avifauna known. The low representation of the Columbiformes in modern (and Holocene) New Zealand is in stark contrast with the diversity of Australia and New Caledonia, which possess 27 species in 11 genera and six species in five genera, respectively. A similar pattern of contrasting diversity among these areas is seen in many other bird groups, including kingfishers and raptors (Trewick & Gibb, 2010).

In this study we generated mitochondrial sequence data in order to examine the population structure of *Hemiphaga*, to measure the scale of diversity within this small genus that is distributed among quite widely spaced oceanic islands, and to place the exchange between island populations in context alongside the evolutionary history of these birds in mainland New Zealand. We consider the scale of genetic diversity and spatial structuring of *Hemiphaga* and other flying birds in the New Zealand region, with respect to biotic assembly.

IV.2 MATERIAL AND METHODS

Sampling

The threat status of *Hemiphaga n. novaeseelandiae* was recently reassessed and although it has been changed from 'declining' in 2005 (Hitchmough *et al.*, 2007) to 'not threatened' in 2008 (Miskelly *et al.*, 2008) as a result of the national population increasing mostly due to intensive predator control (Clout *et al.*, 1995; Innes *et al.*, 2004), these birds are protected by law. They are also sensitive to disturbance as a result of capture and manipulations, so we avoided sampling live birds. However, *Hemiphaga* are prone to collision with vehicles and windows in urban areas and due to their protected status, dead birds are routinely submitted to Department of Conservation (DoC) offices, where they are held in freezers. Our New Zealand sampling therefore reflects the relatively high densities of *Hemiphaga* in several urban areas and the proximity of DoC offices. Accordingly, these samples have little locality data associated with them but they originated from within about 50 km of the DoC office from which they were sourced.

In this study we used muscle tissue from frozen birds. For the population studies, 67 pigeon samples from mainland New Zealand (*Hemiphaga n. novaeseelandiae*) were made available. Six tissue samples of *H. chathamensis* used in this study, which also originated from accidental deaths, were provided by the DoC Chatham Islands area office. Three toepad samples of the extinct Norfolk Island pigeon (*H. n. spadicea)* were kindly provided by the World Museum, Liverpool (D3544) and the Natural History Museum, New York (AMNH 268826 & AMNH 611718). These originated from aviary birds collected in the 1800s. Because *Hemiphaga* on the Kermadec Islands is represented by a single subfossil bone only, we were not able to include this extinct species in our study. The *Lopholaimus antarcticus* (EBU45523M) tissue sample came from the Australian Museum, Sydney.

DNA extraction

For DNA extraction of the modern *Hemiphaga* and *Lopholaimus* samples, the GenElute Mammalian Genomic DNA kit (Sigma, Auckland, New Zealand) was used following the manufacturer's protocol. The DNA extractions of the extinct Norfolk Island pigeon samples were undertaken in a dedicated ancient DNA laboratory, remote from modern DNA facilities, using the Qiagen QiAMP DNA Minikit (Qiagen, Auckland, New Zealand), following standard procedures for ancient DNA (Willerslev & Cooper, 2005).

Polymerase chain reaction and sequencing

We used data from seven mitochondrial genes. Our primary data consisted of DNA sequences from cytochrome *b* (cyt *b*) and the D-loop region, which have both been shown to provide sufficient sequence variation to resolve population structure in pigeons and other birds (e.g. Wenink *et al*., 1994; Johnson & Clayton, 2000; Butkauskas *et al.*, 2008; Zino *et al.*, 2008). D-loop sequences were then employed for our analyses of the radiation within *Hemiphaga n.*

novaeseelandiae and the genus *Hemiphaga.* Initially we amplified a 1291 bp fragment (for details of these and other primers see Table 4.1), but excluded approximately 492 bp adjacent to the 12S rRNA gene because it could not be sequenced reliably due to the presence of 5 or more 52 bp tandem repeats. We also sequenced a 978 bp cyt *b* DNA region. In order to amplify and sequence the D-loop and cyt *b* fragments from DNA extractions of the extinct Norfolk Island pigeon samples, we designed *Hemiphaga*-specific primers using the program Oligo4 (Molecular Biology Insights, Inc., Cascade, CO) to generate a series of short (~130 – 200 bp) overlapping fragments. For additional comparison of the different species of *Hemiphaga* and to assess placement of the root using an outgroup we obtained data from 12S, 16S, ND1, ND2 and cytochrome *c* oxidase subunit I (COI) for *H. chathamensis* using a combination of polymerase chain reaction (PCR) primers. These data supplemented published sequences for the near relatives, *H. n. novaeseelandiae*, *Gymnophaps albertisii* and *Lopholaimus antarcticus* and further outgroup representatives *Ducula melanochroa* and *Ptilinopus luteovirens* (Gibb & Penny, 2010) (see Table 4.2 for GenBank accession numbers).

PCR amplifications for all samples were carried out in 20 µl volumes, using RedHot Taq (AbGene, ThermoScientific, Epsom, UK). The PCR thermal profile for the D-loop started with 94°C for 2 min followed by 25 cycles of: 94°C for 45 s, 50°C for 30 s, and 72°C for 30 s for all reactions. Cyt *b* reactions were run with 35 cycles and 2 min extension at 72°C. The PCR thermal profile of ancient DNA samples for D-loop and cyt *b* was 94°C for 2 min followed by 38 cycles of: 94°C for 30 s, 50°C for 45 s, and 72°C for 2 min for all reactions. PCR reactions using DNA of the extinct Norfolk Island subspecies were prepared in a dedicated ancient DNA laboratory. All PCR products were checked on 1% agarose gels. Each product was sequenced using standard protocols for the ABI Prism BigDye Terminator Ready Reaction Kit (Applied Biosystems, Mulgrave, Australia) and run on an ABI Prism 377 automated sequencer (Applied Biosystems). All sequences were edited using Sequencher software (Gene Codes Corporation, Ann Arbor, MI, www.genecodes.com), and aligned using Se-Al v2.0a11 (Rambaut, 1996). All sequences were deposited at GenBank (GQ912532–912619 and HM165267–165270).

Phylogenetic and population analyses

We examined the placement of the root in *Hemiphaga* using combinations of sequence data and taxa. We used cyt *b* data (978 bp fragment) for a subset of *Hemiphaga* samples representing mainland New Zealand (six specimens), the Chatham Islands (three) and Norfolk Island (one) populations, plus two of the nearest living relatives as an outgroup (*Lopholaimus antarctica* and *Gymnophaps albertisii*) (Pereira *et al.*, 2007). We ran separate analyses using a combined dataset of cyt *b* and D-loop sequences from *H. n. novaeseelandiae* (six specimens), *H. n. spadicea* (one), *H. chathamensis* (three) and *Lopholaimus antarcticus* (one), and a six gene data seta (12S, 16S, ND1, ND2, COI, cyt *b*) with *H. n. novaeseelandiae*, *H. chathamensis*, *Ducula melanochroa*, *Gymnophaps albertisii*, *Lopholaimus antarcticus* and *Ptilinopus luteovirens* (see Table 4.2). MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) was used to implement Bayesian

Table 4.1: Table of primers designed for and used in this study. Primers denoted with * were taken from the primer database of the Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Palmerston North and were designed, modified and/or improved by various researchers. Alphabetic codes indicate source references where applicable: a) Cooper *et al.*, 2001; b) Sorenson *et al.*, 1999. To amplify and sequence cytochrome *b* (Cyt *b*) and D-loop of *Hemiphaga novaeseelandiae spadicea*, new primers (Liv_) were designed for this study.

Primer name	Sequence	Gene
Av6335tTrpF*[a]	GGCCTTCAAAGCCTTAAATAAGAG	COI
Bat6871tserR*	GTTCGATTCCTTCCTTTCTT	COI
Av1753F12S*[a,b]	AAACTGGGATTAGATACCCCACTAT	12S
16S2919R*[a]	GTTGAGCTTTGACGCACTC	12S
Av2703F16S*	GACTTGTTAGTAGAGGTGAAAAGCC	16S
Av4015tLeuR*	GGAGAGGATTTGAACCTCTG	16S
Av3787F16S*[a]	CGATTAACAGTCCTACGTG	ND1
Av5201tMetR*[a]	CCATCATTTTCGGGGTATGG	ND1
DG6590R*	TGTTGAGGGAAGAATGTTAG	ND2
Av438F DloopB *	TCACGTGAAATSAGCAACCC	D-loop
Av1301R12S *[a]	CAGTAAGGTTAGGACTAAGTC	D-loop
Av15107CytbF *	CATCCGTTGCCCACACATGYCG	Cyt *b*
Av16137tProR *	ARAATRCCAGCTTTGGGAGTTGG	Cyt *b*
Liv_175F	GGAGCCACAGTCATTACCAACC	Cyt *b*
Liv_323R	GTCTCACCATCATCCACCTCAC	Cyt *b*
Liv_264F	CAACCCCACACTAACACGATTC	Cyt *b*
Liv_405R	CCCATTCCACCCTTACTTTTCC	Cyt *b*
Liv_386F	CTTCCAACTGCGACAAAATCC	Cyt *b*
Liv_539R	TCACACCCCCTCATATTAAGCC	Cyt *b*
Liv_514F	AACTTCACACCAGCAAACCCAC	Cyt *b*
Liv_664R	CCCCTGCTCCACAAGTCTAAAC	Cyt *b*
Liv_665F	CCCTGCTCCACAAGTCTAAAC	Cyt *b*
Liv_817R	CTCACCTACTTCACTATCCTCC	Cyt *b*
Liv_813F	CTCCCTCACCTACTTCACTATC	Cyt *b*
Liv_947R	CCCCTTCTTAGAGTTTCC	Cyt *b*
129_pigeonF	CCATTTTAGTCCGTGATCGC	D-loop
130_pigeonR	CGCGATCACGGACTAAAATG	D-loop
317_pigeonR	AGTGCATCAGTGTAAAGGTG	D-loop
320_pigeonF	CTTTACACTGATGCACTTTG	D-loop
456_pigeonF	TTAATGAATGCTTGGAGGGC	D-loop
661_pigeonR	GTTGGAGGAGTTTTTAGAGG	D-loop

References:
Cooper, A., Lalueza-Fox, C., Anderson, S., Rambaut, A., Austin, J. & Ward, R. (2001) Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature,* **409**, 704-707.

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T. & Mindell, D. P. (1999) Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution*, **12**, 105-114.

Table 4.2: Additional species and genes included in this study with GenBank accession numbers, from Gibb & Penny, 2010. X denotes that the respective gene was not available for analysis.

Species	12S	16S	ND1	ND2	COI	Cyt *b*
Ducula melanochroa	GU230689	GU230689	GU230689	GU230691	GU230692	GU230690
Gymnophaps albertisii	EF373303	X	X	EF373337	EF373375	EF373280
Hemiphaga n. novaeseelandiae	EU725864	EU725864	EU725864	EU725864	EU725864	EU725864
Lopholaimus antarcticus	GU230702	GU230702	GU230701	GU230698	GU230700	GU230699
Ptilinopus luteovirens	GU230707	GU230707	GU230706	GU230703	GU230705	GU230704

Reference:
Gibb, G. C. & Penny, D. (2010) Two aspects along the continuum of pigeon evolution: A South-Pacific radiation and the relationship of pigeons within Neoaves. *Molecular Phylogenetics and Evolution*, **56**, 698-706.

analyses using models of DNA evolution indicated using jModelTest 3.5 (Guindon & Gascuel, 2003; Posada, 2008). However, we encompassed the different models selected using alternative criteria in jModelTest by repeating the analyses using either GTR+I+Γ or HKY+I. For each data set replicate analyses were employed to ensure convergence, each consisting of two independent Markov Chain Monte Carlo (MCMC) runs of six to ten million generations, applying a burn-in of 10-20%, after consideration of output statistics. We referred to the average standard deviation of split frequencies and potential scale reduction factors, which were accepted if less than 0.01 and at or near 1.000 respectively (Gelman & Rubin, 1992; Ronquist *et al*., 2005). For analysis of multigene data with MrBayes 3.1.2 we partitioned the dataset by gene and unlinked values of model parameters. Tree topologies from alternative methods and models of DNA evolution were scrutinized and node support statistics compared. To further examine node support in the cyt *b* tree we used maximum parsimony (MP) analysis implemented in paup* (Swofford, 1998) with stepwise addition of the starting tree, tree bisection–reconnection, all characters given equal weights and bootstrapping with 1000 replicates.

Haplotype diversity (*h*), nucleotide diversity (π, Nei, 1987), Tajima's *D* (Tajima, 1989) and the average number of nucleotide differences (*k*) were calculated using DnaSP v4.0 (Rozas *et al.*, 2003). Tajima's *D* statistic (Tajima, 1989) was developed to distinguish homologous DNA sequences evolving in a non-random manner (i.e. lack of neutrality). However, it has been shown that for neutral markers such as the mitochondrial DNA (mtDNA) D-loop, the statistic provides a useful indicator of population range expansion and exchange (Ray *et al.*, 2003; Wegmann *et al.*, 2006). We calculated pairwise F_{ST} values for the nine regional populations using Arlequin v3.0 (Excoffier *et al.*, 2005).

Geographical structure was investigated using a parsimony haplotype network of mainland New Zealand *Hemiphaga* D-loop sequences constructed with the program TCS 1.21 (Clement *et al.*, 2000). For this analysis New Zealand was divided into three main sampling areas, north, central and southern, each with three subsamples (figure 4.3). More detailed structuring of populations was not possible, because of the lack of precise location data of the samples (see *Sampling*). The analysis was run using a 95% connection limit.

Nested clade analysis (NCA; Templeton, 1998) is a network-based phylogeographical analysis applicable to intraspecific data (Schultheis *et al*., 2002) and data from closely related species (Abbott & Double, 2003). Despite some criticism about the sensitivity and limitations of nested clade phylogeographic analysis (NCA) (Petit, 2008), it remains a valuable means to explore phylogeographic structure and is useful in revealing major subdivisions. Although originally developed with mitochondrial DNA genealogical data in mind the method is now recognized as likely to be most reliable when applied to multiple (cross validating) loci (Garrick *et al.*, 2008; Templeton, 2008*a*), it was here cautiously applied and in combination with other measures of population structure. A nested series of clades within the network was identified using the algorithm of Templeton & Sing (1993) and Crandall (1996) and followed by statistical analyses of the geographical data using the software GeoDis (Posada *et al*., 2000). All statistical analyses in GeoDis were performed using 1,000 random permutations. The statistics calculated for all clades were: (i) clade distance (D_C), measuring the average distance of all haplotypes in the clade from the geographical centre of distribution; (ii) nested clade distance (D_N), which measures how widespread a particular clade is relative to the distribution of other clades in the same nesting clade; (iii) interior-tip distances (Int_C and Int_N). This interior vs. tip contrast of clades corresponds to younger / rare clades (tip clade) relative to their ancestors'/ common clades (interior clades) (Crandall & Templeton, 1993). Then testing for significantly small or large D_C or D_N distances in each nested clade was used to see whether the null distribution of no association between haplotype distributions and geography could be rejected. The revised inference key of Templeton (2008*b*) was used to interpret the population genetic and evolutionary implications of the results.

An unrooted neighbour-net network was constructed with D-loop sequences using SplitsTree 4.8 (Huson & Bryant, 2006) with default settings to show the relationships within the genus *Hemiphaga*, and the extent of conflicting signal among these data. In population studies where sequences are naturally very similar to each other it is necessary to provide evidence that the tree found is the shortest possible under parsimony criteria. We employed the program MinMax Squeeze (Holland *et al.*, 2005), which compares the shortest tree found with the shortest tree possible on the data, to determine this with the *Hemiphaga n. novaeseelandiae* population dataset.

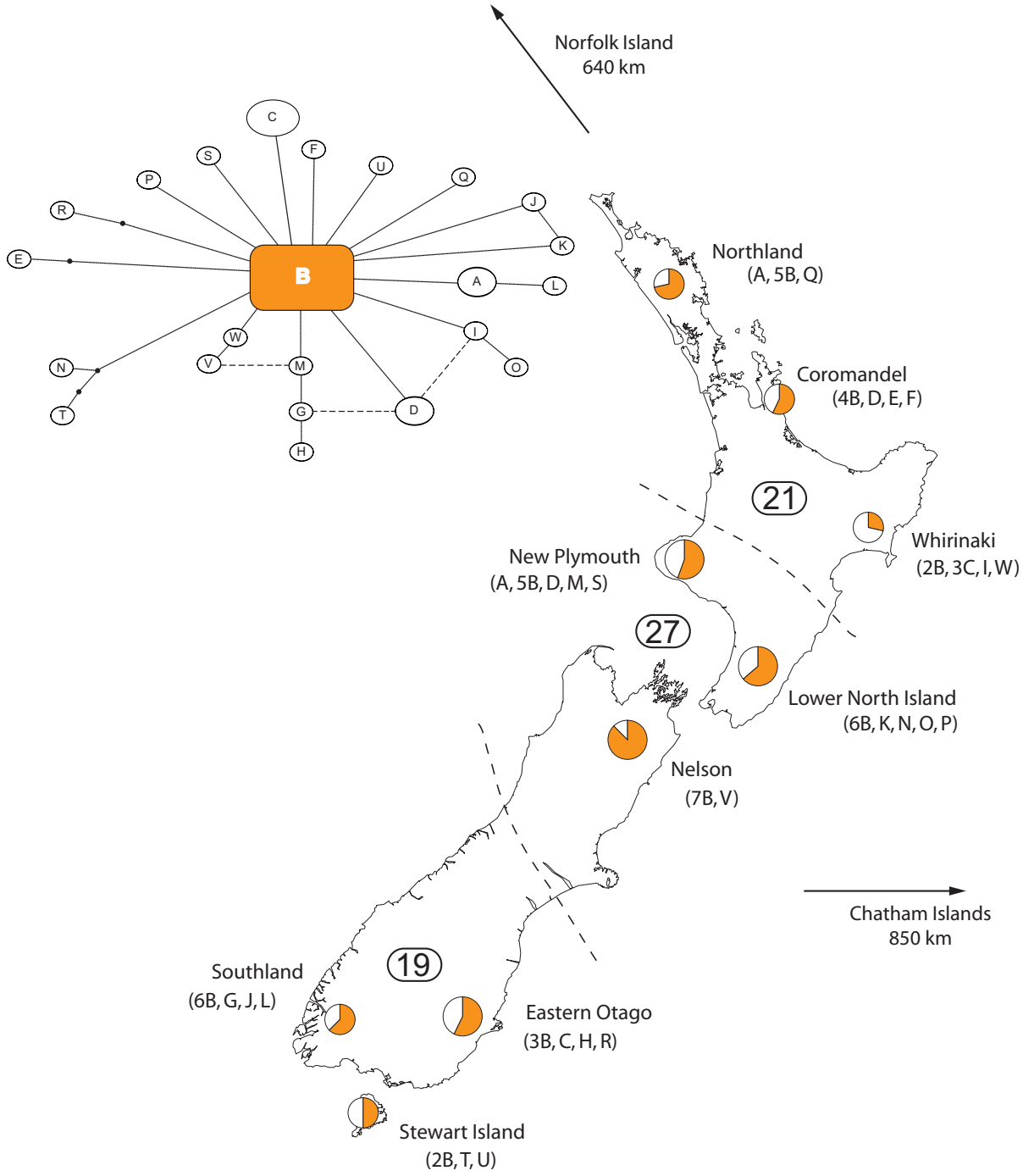


Figure 4.3: Map of New Zealand showing the three main sampling areas, divided by dotted horizontal lines. The numbers in ovals represent the summed sample size per main sampling area. Letters represent different haplotypes of *Hemiphaga novaeseelandiae novaeseelandiae* within each subpopulation (see inset haplotype network and Table 4.3). Pie charts depict the frequency of the most common haplotype B (in orange) per subpopulation in the mainland New Zealand woodpigeon (*Hemiphaga novaeseelandiae novaeseelandiae*) population.

IV.3 RESULTS

Preliminary Bayesian analysis using six mitochondrial genes and an outgroup representing four genera confirms the placement of *Hemiphaga* nearer to *Lopholaimus* and *Gymnophaps* than *Ducula* and *Ptilinopus* (figure 4.4a). A tentative tree hypothesis based on maximum parsimony (MP) and Bayesian analyses returned trees for cyt *b* that consistently placed the root between *H. chathamensis* and the two *H. novaeseelandiae* lineages, making New Zealand and Norfolk Island lineages monophyletic (figure 4.4b). A tree of the same topology was obtained using a combined cyt *b*/D-loop data set (not shown). Not surprisingly, node support for the split between mainland New Zealand and Norfolk Island *Hemiphaga* was intermediate (MP 0.78, MrBayes 0.55) given the disparity between low ingroup diversity and distance to the nearest common ancestor. This tentative rooting is consistent with the current taxonomic treatment of the genus and thus with the current morphological evidence and inference from genetic distance data. An unrooted neighbour-net network (figure 4.4c) of *Hemiphaga* D-loop data revealed a low level of conflicting signal within the ingroup and a relatively long branch between *H. chathamensis* and the pairing of *H. novaeseelandiae* subspecies, and this is consistent with placement of the root between the two species.

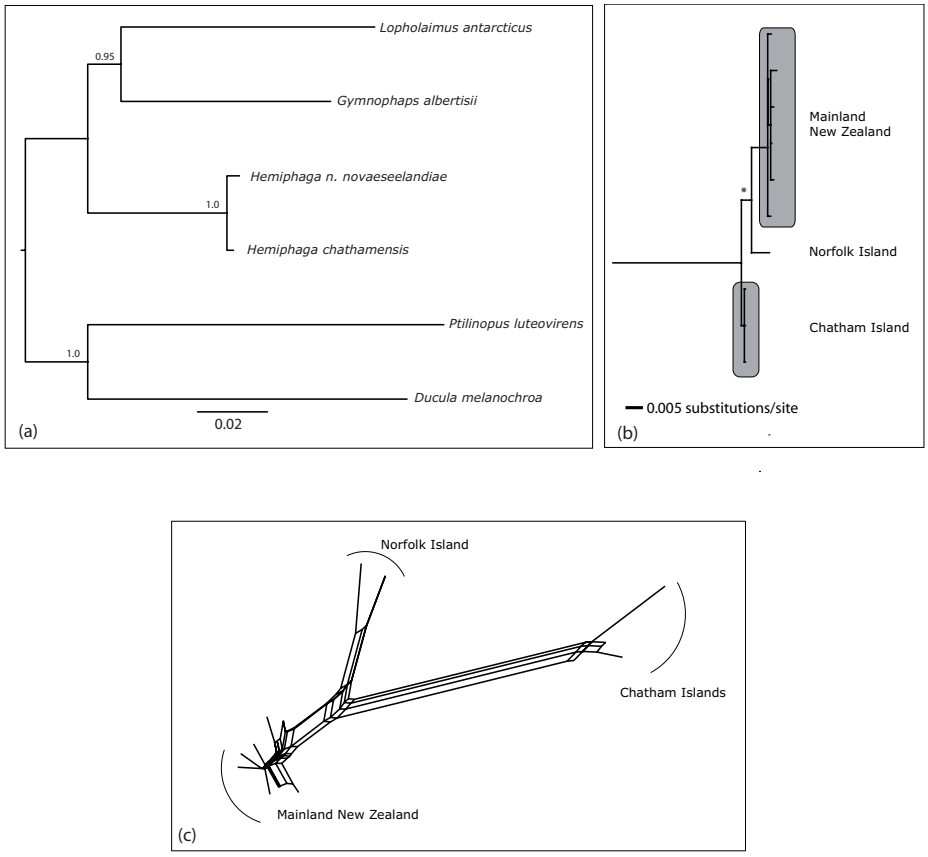


Figure 4.4: (a) A six-gene Bayesian tree of *Hemiphaga novaeseelandiae novaeseelandiae*, *H. chathamensis* and their closest allies; values on branches are Bayesian posterior probabilities. (b) A cytochrome *b* tree of the genus *Hemiphaga* rooted with closest sister taxon *Lopholaimus* (not shown); the asterisk denotes the node with available values: 0.55 Bayesian posterior probability and 0.75 maximum parsimony bootstrap value (1000 replicates). (c) An unrooted D-loop neighbour-net splitsgraph of the genus *Hemiphaga* depicting the conflict in the data set.

We assessed the likely time frame of *Hemiphaga* diversification by comparison of genetic distances with published studies of other birds. All genetic distances among *Hemiphaga* samples were low by comparison to published values for other birds. Indeed, we found no sequence divergence at the COI locus used widely in "DNA barcoding" studies (e.g. Hebert *et al.*, 2004; Kerr *et al.*, 2007).

The Chatham Islands population shares no haplotypes (D-loop or cyt *b*) with the mainland New Zealand population. With the present sampling, the maximum uncorrected genetic distance in D-loop among the Chatham haplotypes is 0.005, and there is a mean uncorrected genetic distance of 0.028 to the mainland species. Cyt *b* sequences differed between the Chatham Islands and mainland New Zealand by 0.012, more or less identical to the ~0.01 difference reported by Millener & Powlesland (2001).

We were also able to obtain D-loop sequences from three specimens of the extinct *H. n. spadicea* from Norfolk Island, which provide valuable comparison to the mainland New Zealand data. Although diversity is not expected to be high on a small island, the Norfolk Island specimens express two different haplotypes in the D-loop fragment. They show mean uncorrected genetic distances of 0.014 to mainland New Zealand specimens and 0.029 to the Chatham Island species, implying a younger split from New Zealand than for the Chathams' population. Additionally, we sequenced the cyt *b* fragment for one Norfolk Island specimen. The mean uncorrected genetic distance to the Chatham Island species was 0.011 and 0.013 to the mainland species.

Comparison of D-loop sequences (733 bp) from 67 specimens of *Hemiphaga n. novaeseelandiae* revealed 23 different haplotypes within mainland New Zealand, of which 19 were singletons, and additionally four different haplotypes from the Chatham Islands and two from Norfolk Island were found (Table 4.3). A statistical parsimony network was generated in TCS containing all mainland New Zealand D-loop sequences (inset figure 4.3). Among the mainland New Zealand samples the most common haplotype (B) was found at least twice in each of the nine sampled subpopulations, but only three other haplotypes (A, C, D) were found in more than one location (figure 4.3). The relative abundance of the common haplotype B, compared to rare haplotypes in each subpopulation, indicates the dominance of haplotype B. Haplotype C was found four times, thrice in a northern (Whirinaki) and once in a southern (Eastern Otago) subpopulation. Haplotypes A and D were found twice each, in northern and central populations. Nineteen other haplotypes were encountered just once each and most of these differed by just one nucleotide substitution from the common haplotype B (inset figure 4.3). Even directly connected haplotypes show no spatial distribution (e.g. haplotypes M, G and H occur in New Plymouth, Southland and Eastern Otago) (figure 4.3).

Populations of *Hemiphaga n. novaeseelandiae* displayed very low levels of DNA nucleotide diversity within mainland New Zealand (Table 4.4). The sequences of the northern area had the highest level of haplotype and nucleotide diversity, followed by the southern and central populations (Table 4.4). Coalescent theory predicts that older (interior) haplotypes are likely to be more common than derived (tip) haplotypes. Tip haplotypes are defined as those

Table 4.3: D-loop haplotype frequency in *Hemiphaga* populations and regions of mainland New Zealand; n = number of individuals sequenced per population; N_{haps} = number of different haplotypes within sampled areas; haplotype codes as in figures 4.3 & 4.5. A total of 67 mainland New Zealand (*H. novaeseelandiae novaeseelandiae*), six Chatham Islands (*H. chathamensis*) and three Norfolk Island (*H. novaeseelandiae spadicea*) birds were assessed.

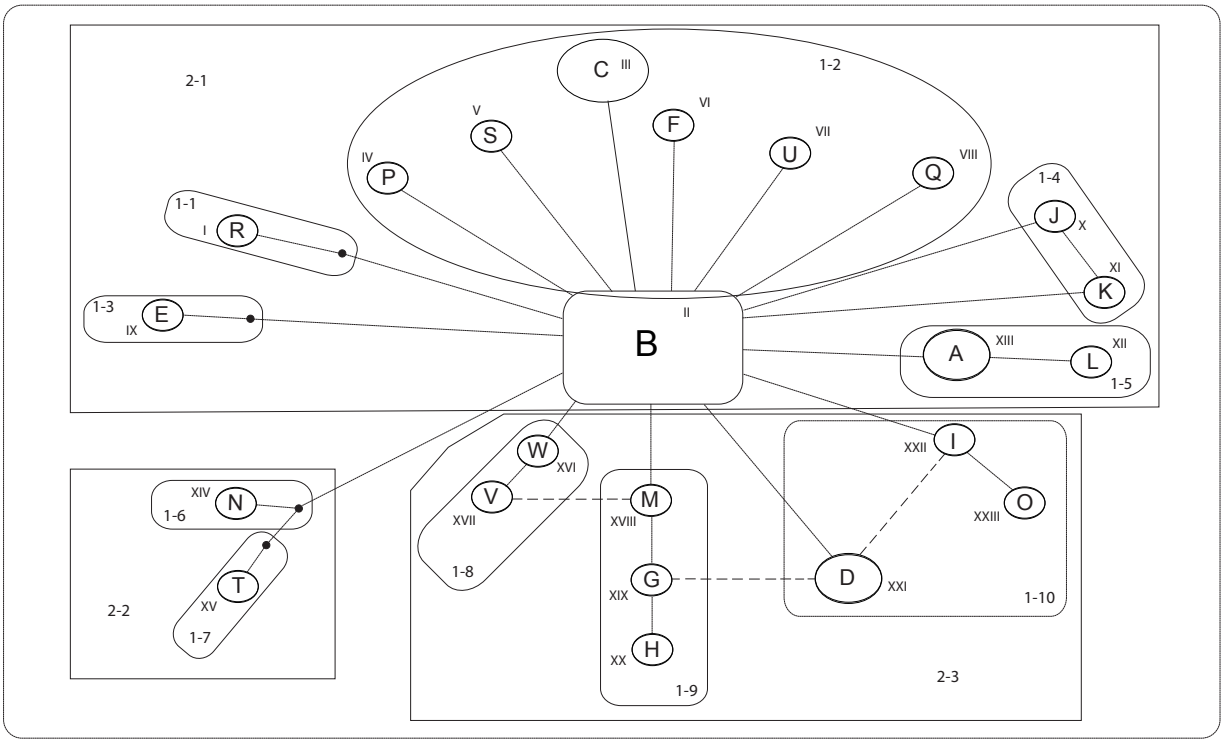
Population	Region	n	N_{haps}	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	a	b	c	d	e	f
Northland	N	7	3	1	5															1												
Coromandel	N	7	4		4		1	1	1																							
Whirinaki	N	7	4		2	3						1														1						
New Plymouth	C	9	5	1	5		1									1						1										
Lower North Island	C	10	5		6									1			1	1	1													
Nelson/Blenheim	C	8	2		7																				1							
Eastern Otago	S	6	4		3	1					1										1											
Southland	S	9	4		6					1			1		1																	
Stewart Island	S	4	3		2																		1	1								
Northern	N	21	9	1	11	3	1	1	1			1								1						1						
Central	C	27	10	1	18		1							1		1	1	1	1			1			1							
Southern	S	19	9		11	1				1	1		1		1						1		1	1								
All New Zealand		67		2	40	4	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1						
Chatham Islands		6	4																								2	2	1	1		
Norfolk Island		3	2																												2	1

connected to only one other haplotype, whereas interior haplotypes are connected to multiple haplotypes (Crandall & Templeton, 1993). A statistical parsimony network, generated in TCS containing all mainland New Zealand D-loop sequences, showed that the B haplotype is the most common haplotype and is connected to multiple others (figure 4.5). The nested clade design and clade numbers are given in figure 4.5. Results of the NCA calculation are given in figure 4.6 and chains of inference for each clade with significant geographical associations are listed in Table 4.5. The NCA analyses based on the TCS network for test of non-random association of haplotypes indicated only three clades for which results about demographic history can be drawn using this method (Table 4.5 and figure 4.5). Haplotypes of clade 1-2 (including samples from every population within New Zealand) are suggested to have been connected by 'restricted gene flow with isolation by distance', meaning limited gene flow between distant populations. For haplotypes of clade 2-1, the inference key suggests 'restricted gene flow/ dispersal but with some long distance dispersal'. The haplotypes in clade 1-9, found in central and southern populations, are inferred as having separated by 'allopatric fragmentation' in the past. For the total cladogram the inference key was not able to explain the relationship between all haplotypes, but suggests that there is 'no geographic association of haplotypes' which could be due to small sample size or inadequate geographical sampling of mainland New Zealand *Hemiphaga*.

Table 4.4: DNA variation and haplotype diversity within and between regional samples of *Hemiphaga novaeseelandiae novaeseelandiae* in New Zealand, with the sample size for each region (n), number of observed haplotypes (Nhaps), average number of nucleotide differences (k), nucleotide diversity (π x10^{-3}), number of polymorphic sites (S), Tajima's D ($P<0.05$) and haplotype diversity (h).

Area	*n*	N_{haps}	*k*	π (± SD)	*S*	Tajima's *D*	*h*
Northern	21	9	1.011	1.55 (± 0.34)	8	-1.815	0.719
Central	27	10	0.872	1.34 (± 0.35)	9	-1.996	0.564
Southern	19	9	0.936	1.44 (± 0.43)	10	-2.126	0.614
Total population	67	23	0.925	1.42 (± 0.36)	16	-2.141	0.623
Northern/Central			0.934	1.43 (± 0.36)			
Central/Southern			0.973	1.38 (± 0.38)			
Northern/Southern			0.967	1.48 (± 0.39)			

Pairwise F_{ST} comparisons (results not shown) revealed no significant difference in the genetic composition of the nine regional sample locations in *Hemiphaga* in mainland New Zealand. Tajima *D* tests (Tajima, 1989) for the three separate mainland New Zealand populations of *H. n novaeseelandiae* and the complete mainland New Zealand dataset were consistent with range expansion with high levels of migration among demes (Table 4.4). Negative Tajima's *D* statistics for the different populations indicate an excess of sites with low frequency polymorphisms, i.e. the population has yet to reach equilibrium (Tajima, 1989).



Total cladogram

Figure 4.5: Nested clade design of the haplotype network of mainland New Zealand *Hemiphaga* D-loop sequences generated with TCS (Clement *et al.*, 2000). Circles indicate haplotypes sampled, small black circles depict unsampled/extinct haplotypes and lines between haplotypes indicate single nucleotide substitutions. Letters inside circles represent different haplotypes and correspond to Table 4.3 & figure 4.3. Numbers and boxes represent clades defined according to NCA (Templeton, 1998).

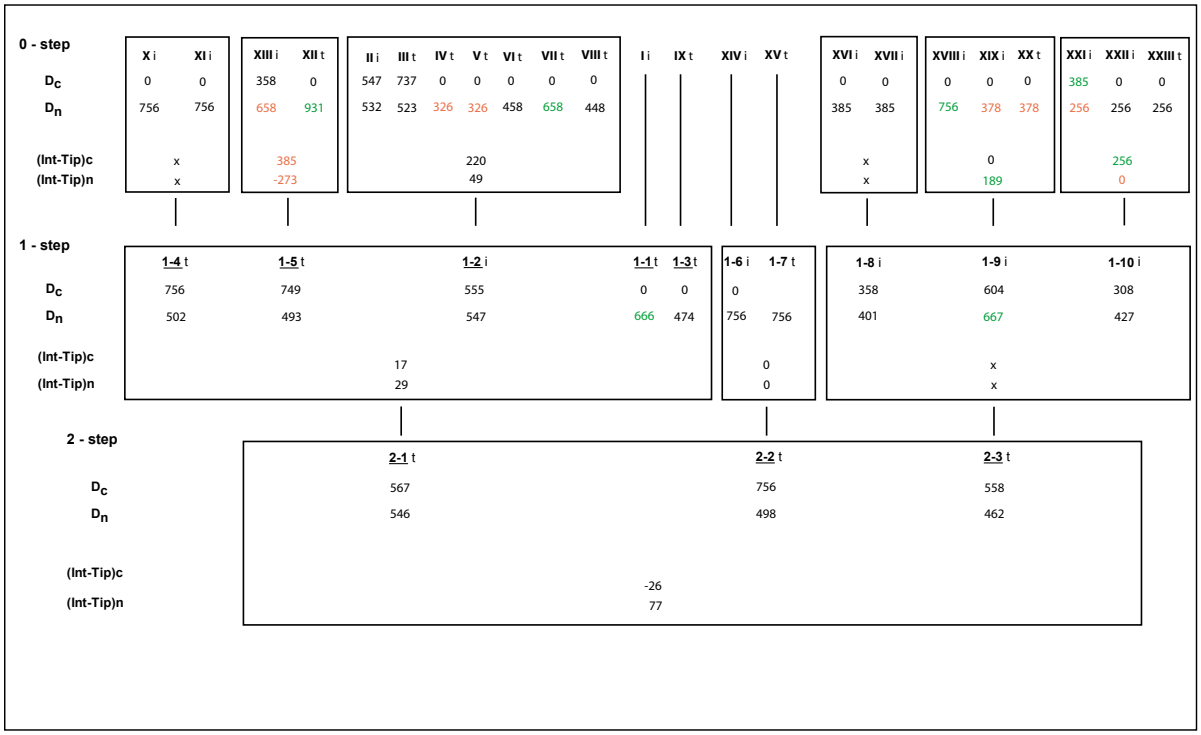


Figure 4.6: Result of the nested clade analysis of geographical distances for the mainland New Zealand *Hemiphaga* D-loop sequences. Clade levels increase from top to bottom. The small letters i and t refer to "interior" and "tip" positions respectively. Haplotypes nested in a one-step clade are grouped in boxes following the nested design shown in figure 4.5. In each box, clade distances (Dc) and nested clade distances (Dn) calculated for each clade within the nested group are shown as well as the average difference in distance between interior clades [(Int-Tip)c] and tip clades [Int-Tip)n] for Dc and Dn respectively. Significantly large or small distance values (5% level) are coloured, with large values shown in green and small values in orange.

Table 4.5: Interpretation of the results of the NCA cladogram (figures 4.5 & 4.6) using the inference key of Templeton (2008*b*).

Nested Clade	Permutational $\chi 2$ statistics	*p*	D_c	D_n	Inference chain	Inferred geographical pattern
1 - 2	14.39	0.14	554.16	547.33	1-2-11-17-4-NO	Restricted gene flow with isolation by distance
1 - 5	3.0	1.0	749.0	493.30	1-19-20-NO	Inadequate geographical sampling
2 - 1	5.93	0.87	567.23	546.29	1-2-3-5-6-7-YES	Restricted gene flow / dispersal but with some long distance dispersal
1 - 9	3.0	1.0	604.80	667.43^{L}	1-2-11-17-4-9-NO	Allopatric fragmentation
1 - 10	2.0	1.0	308.00	427.93	1-2-3-5-6-7-8-NO	Sampling design inadequate to discriminate between isolation by distance vs. long distance dispersal
2 - 3	5.63	0.33	558.19	462.00	1-2 (inconclusive outcome)	
Total Cladogram	1.17	0.95				No geographic association of haplotypes

Analyses with MinMax Squeeze (Holland *et al.*, 2005) demonstrated that no shorter tree was possible for the mainland New Zealand D-loop sequence data, and for the data that included sequences from Norfolk Island and the Chatham Islands. For the mainland New Zealand samples, this means that the parsimony tree is almost certainly the maximum likelihood tree because the tree consists almost entirely of sequences linked by no more than a single change, and in such cases the parsimony tree is the maximum likelihood estimator (Steel & Penny, 2000).

IV.4 DISCUSSION

The large forest pigeon *Hemiphaga n. novaeseelandiae* is distributed throughout mainland New Zealand where suitable habitat exists today. We found that genetic diversity at the mtDNA D-loop locus lacks spatial structure. The pattern of low diversity, shallow coalesence and high connectivity among populations of *Hemiphaga* within mainland New Zealand is indicative of a recent bottleneck and rapid recent range expansion. The geophysical phenomenon most likely to have resulted in substantial population reductions and thus genetic bottleneck that could yield this pattern is Pleistocene climate cooling/aridification during global glacial phases. Similar patterns on a larger spatial scale have been identified among birds in Europe (Merilä *et al.*, 1997), North America (Zink, 1996) and Australia (Joseph & Wilke, 2007). In New Zealand, palynological studies indicate that the primary habitat (forest) of *Hemiphaga* was much reduced in total area and distribution during the Last Glacial Maximum, and probably also during prior glaciations (McGlone, 1985; Alloway *et al.*, 2007). The most extensive forest patches are thought to have been in northern New Zealand (McGlone *et al.*, 2001; Alloway *et al.*, 2007) and this might explain the slightly higher haplotype diversity in northern populations apparent in our sample (Table 4.4). The modern widespread distribution of *Hemiphaga* in mainland New Zealand (despite recent human interference) confirms subsequent range expansion, and the near

continuous extent of forest habitat in New Zealand immediately prior to human contact (Trewick & Morgan-Richards, 2009) would have provided ample opportunity for gene flow throughout and among the three main islands as these birds are not philopatric. Field studies confirm that *Hemiphaga* pigeons are highly effective flyers that regularly travel substantial distances to reach their food resources, including crossing sea straits between islands of mainland New Zealand (Powlesland *et al.*, 2007).

This pattern of population structure is not unique to *Hemiphaga* among New Zealand birds, suggesting a more general response of expansion out of refugia in the wake of expanding forest habitat. Similar low nucleotide diversity combined with high haplotype diversity is documented in the kokako (*Callaeas cinerea*, Murphy *et al.*, 2006) and New Zealand robin (*Petroica australis*, Miller & Lambert, 2006). On the other hand kiwi (*Apteryx*), a group of ground-dwelling birds, comprises deeper phylogeographic and taxonomic diversity consistent with regional persistence of populations through the Pleistocene (Baker *et al.*, 1995; Burbidge *et al.*, 2003), perhaps explained by a lesser dependence on high forest.

Although the process of population retraction and expansion in New Zealand could have occurred repeatedly throughout the Pleistocene, we see no signature of earlier episodes or survival of multiple mainland lineages or discrete populations through the Pleistocene. There is, however, spatial structuring of genetic diversity among the more widely spaced islands (New Zealand, the Chatham Islands, Norfolk Island) even though total genetic diversity is still very low. Colonization of the Chatham Islands cannot have occurred before 4 Ma, as geological evidence indicates that the Chatham archipelago was completely submerged prior to this time (Campbell *et al.*, 2006; Campbell & Hutching, 2007; Trewick *et al.*, 2007). This nevertheless provided ample time for the morphological and genetic separation of *H. chathamensis*. The D-loop variation we found in a small Chatham Island sample indicates that this species maintained relatively high genetic diversity on this small archipelago (970 km^2 total land area) despite recent population decline. This population went through at least one well documented bottleneck in the 1980s, with a maximum of 50 birds surviving mostly in a small area of southern Chatham Island (Grant *et al.*, 1997). Norfolk Island also formed in the late-Pliocene (3.2–2.4 Ma) by intraplate volcanism (Johnson, 1989), so here, as with the Chatham Islands, the biota must have assembled predominantly through recent colonization over water. The slightly closer affinity of the Norfolk Island pigeon to the New Zealand taxon (compared to the Chatham birds) is consistent with a more recent exchange between mainland New Zealand and Norfolk Island, but it is not possible to determine the direction of exchange with the present data.

Although the Chatham Islands and Norfolk Island are Pliocene in age, colonization by *Hemiphaga* of these islands could have occurred at any time after their formation and the establishment of suitable vegetation. These islands are, and have always been, separated from mainland New Zealand by more than 600 km of ocean, yet both have been colonized during their short history by a range of forest birds, including *Cyanoramphus* parakeet, *Nestor* parrot, *Petroica* robin, *Chrysococcyx* cuckoo, *Gerygone* warbler, and *Zosterops* silvereyes

(Clements, 2007). The Kermadec Islands, which were also once inhabited by *Hemiphaga*, are even further (>900 km) from New Zealand, and their general lack of bird endemicity has been ascribed to extinction through repeated, violent volcanic eruptions rather than failure of colonization (Worthy & Holdaway, 2002). Each island population represents the descendants of an independent oversea colonization event, replicated by many taxa. Clearly, dispersal over inhospitable habitat is not rare in biogeographic terms.

For *Hemiphaga*, episodes of dispersal between mainland New Zealand, the Chatham Islands and Norfolk Island are likely to have occurred in the Pleistocene rather than earlier in the Pliocene. We found maximum genetic distances among *Hemiphaga* of 1.3% at the cyt *b* locus, which is less than that found among *Cyanoramphus* parakeets (max 2.7% – Boon *et al.*, 2001*b*; Boon *et al.,* 2008), and *Petroica* robins (max 8.7% – Miller & Lambert, 2006) in the same region. Even within mainland New Zealand, cyt *b* genetic diversity of *Petroica* robins is higher (2.7%) than that found in all *Hemiphaga* (Miller & Lambert, 2006). By comparison, a study of doves in North America, known to have been influenced by Pleistocene climate cycling, found intraspecific cyt *b* divergence up to 0.1%, and 0.9% between species (Johnson & Clayton, 2000). This has been interpreted as indicating a phylogenetic split within the last 450,000 years (based on a 2% molcular clock calibration – Johnson & Clayton, 2000). Not surprisingly we found inter- and intraspecific divergences within *Hemiphaga* at the D-loop locus to be slightly higher than for cyt *b*, with a maximum of 2.9% and 1.4%, respectively. Again *Petroica* robins have a higher genetic diversity in D-loop within New Zealand (max 6.4%) and much higher (22.6%) between mainland New Zealand and Chatham Island species (Miller & Lambert, 2006). The same is apparent in *Cyanoramphus* parakeets, with an intraspecific divergence in mainland New Zealand of 3.11% and a maximal interspecific divergence of 9.82% (Boon *et al.*, 2001*a*,*b*). Furthermore, at the COI mtDNA locus, where divergence within bird species is documented as ranging from 0.4 to 5.4% (e.g. Aliabadian *et al.*, 2009; Kerr *et al.*, 2009; Johnsen *et al.*, 2010) with a commonly employed threshold for maximum intraspecific divergence of 2.5% (Hebert *et al.,* 2004), we found no differences between the two *Hemiphaga* species. Together with the young age of the islands, our data indicate extensive mixing in New Zealand and very shallow genetic distances overall and reveal a pattern of recent widespread dispersal.

Although the depth of diversification within *Hemiphaga* does not directly inform on the timing of origin of the lineage in the New Zealand region, the fact that the entire extant diversity is distributed across islands spanning 1200 km of ocean is revealing. Clearly, *Hemiphaga* has retained high mobility even over wide expanses of sea, and has suffered extensive extinction in recent times. The success of *Hemiphaga* in terms of its persistence must rest partly on this ability, so we are left with the conundrum of why the genus is not represented by more and deeper diversity in the New Zealand region and/or further afield in Australia, New Caledonia or other Pacific Islands. Similarly, why has New Zealand no other extant native pigeons, when other genera exist as close as Norfolk Island? The lack of *Hemiphaga* elsewhere might be explained by extinction following human habitat modification or earlier replacement by other dove radiations, including *Ptilonopus* and *Ducula* (Steadman, 1997; Gibbs *et al.*, 2001; Steadman,

2006). Molecular phylogenetics indicate that *Hemiphaga* forms a clade with other species-poor genera (*Lopholaimus*, *Gymnophaps*) but is allied to species-rich genera in Melanesia and Polynesia (*Ptilonopus* and *Ducula*) (Goodwin, 1960; Johnson, 2004; Pereira *et al.*, 2007; Gibb & Penny, 2010). The lack of diversity in *Hemiphaga* and allies may be due to lineage pruning, but it is not possible to determine the timing of this process. Any biogeographic inference drawn from the long *Hemiphaga* branch must be tenuous, because it is not possible to exclude (or estimate the likelihood of) a close relative having existed recently outside the region. Even the recent discovery of a pigeon fossil (single humerus) from early-mid Miocene (19–16 Ma) New Zealand, that may be allied to the *Hemiphaga–Lopholaimus* group (Worthy *et al.*, 2009), does not demonstrate continued occupation of New Zealand by the lineage since that time. Instead, along with other fossils from the Miocene representing taxa not present in New Zealand now, it suggests subsequent extinction of many lineages. Given that the New Zealand avifauna (and biota in general) has experienced substantial extinction and has been subjected to many arrivals during that time frame, we cannot exclude the possibility that there have been repeated arrivals and extinctions of pigeons, including members of this lineage. Indeed this scenario may help reconcile the various patterns of taxon distribution and endemicity observed among the (avi-) fauna (figure 4.2).

It might be expected that when examining the New Zealand avifauna some dominant pattern of distribution and endemicity (indicating a dominant process) would emerge, but this is not the case. Overlapping but incongruent distribution patterns in the New Zealand area might be best explained by repeated colonization in concert with extirpation in various areas. Extinction of lineages (phylogenetic relatives) at any time prior to the present tends to yield increasingly narrow endemism of remaining lineages and increasingly lengthy branches in phylogenetic trees (Trewick & Gibb, 2010). This combination of localized taxa and deep branching phylogenies, especially when the only remaining taxa are found on an island, can lead to an inference of ancient isolation. However, it is obvious that this pattern is deceptive and open to misinterpretation; understanding the evolutionary history of New Zealand's supposedly ancient biota requires a better understanding of dispersal, colonization and extinction governing its assembly.

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V

Intercontinental island hopping: Colonization and speciation of the grasshopper genus *Phaulacridium* (Orthoptera: Acrididae) in Australasia

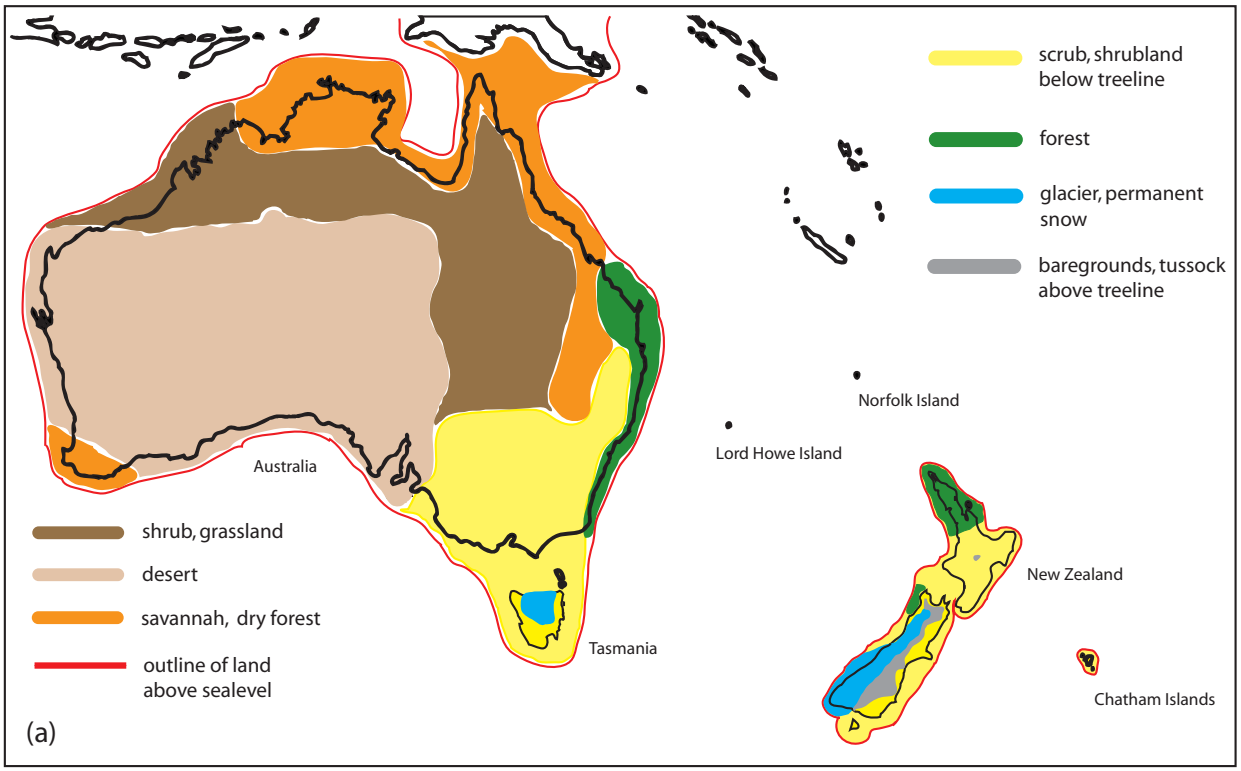
ABSTRACT

Due to their distance from the pole and extent of oceans, sourthern hemisphere lands were not subjected to such severe climatic conditions in the Pleistocene as those in the northern hemisphere. In Australasia, glaciers did develop on the southernmost mountain ranges of New Zealand and Australia (Tasmania). Pleistocene climate cycling did however result in extensive shifts in habitat zones due to fluctuation of rainfall and temperature. Warm and wet conditions during interglacial periods supported forests further south, whereas cool and dry environments during glacial maxima increased the extent of dry grassland and scrub conditions. Such flucutations are likely to have influenced the spatial distribution and evolution of the fauna of Australasia. Phylogeographic patterns in much of the biota of southern New Zealand reveal evidence of geographically structured and genetically divergent lineages indicating survival in refugia during the last glacial maximum. Here we report on the phylogeographic patterns of the grasshopper genus *Phaulacridium* that comprises species inhabiting low altitude grasslands in Australia and New Zealand. We contrast the taxonomic and genetic diversity in these two regions that differ considerably in size and history. The widespread New Zealand species *Phaulacridium marginale* shows a phylogeographic pattern typical of recent range expansion, with low genetic diversity within the species. In stark contrast, the other New Zealand species *P. otagoense* is very localized in two small areas in southern New Zealand but exhibits very high genetic diversity. The phylogeographic patterns in Australian *Phaulacridium* show deeper divergence within the most widespread species, than between different species in the same area. Simple correlations between geographic scale and genetic diversity are not applicable, the data instead implying current ranges of species are poor indicators of their status in the recent evolutionary past.

V.1 INTRODUCTION

Since Darwin (1809-1882) and Wallace (1823-1913), a fundamental objective for biologists has been to understand the diversity and distribution of biota. This endeavour received a substantial stimulus from phylogeographic approaches (Avise *et al.*, 1987; Avise, 2000) which continue to develop and improve (Hickerson *et al.*, 2010). The most extensive phylogeographic studies have been conducted in North America and Europe, where the vast number of studies has provided a degree of consensus on patterns and processes (e.g. Taberlet *et al.*, 1998; Hewitt, 1999, 2000, 2004; Schmitt, 2007). Phylogeographic structuring in these landscapes has been predominantly influenced by two extrinsic factors. The first is the impact of extreme climate cycling throughout the Quaternary period with extension of polar ice sheets, vast periglacial regions with permafrost and lower global temperatures during glacials. The second is the largely continuous distribution of land in northern latitudes across thousands of kilometres of longitude. The biology of northern hemisphere regions to a large extent is a story of dispersal and range shifting since the last glacial maximum (LGM). In the southern hemisphere, the situation is rather different. There was no (significant) extension of polar ice sheets to continents beyond Antarctica, although some small adjacent islands were affected (McIntosh *et al.*, 2009; Neville *et al.*, 2010). Furthermore, land masses are widely spaced and separated around longitude by wide expanses of ocean, which potentially limit dispersal of terrestrial organisms, and also ameliorate the local intensity of climate fluctuations. The Australasian region therefore provides a strong and intriguing contrast to Northern Hemisphere systems, as it includes land areas that differ extensively in size and degree of spatial isolation, ranging from the continent of Australia (7.6 million km^2) in the west to the Chatham Islands (900 km^2) in the east (figure 5.1). During the LGM glaciation was extensive on the Southern Alps in South Island, New Zealand (figure 5.1a & b(*i*)) (Alloway *et al.*, 2007). Australia on the other hand remained largely unaffected, but experienced with New Zealand a general aridification and cooler climate resulting in extensive expansion of shrub and dry grassland and restriction of high forest (figure 5.1a) (Lancashire *et al.*, 2002; McKinnon *et al.*, 2004; Williams *et al.*, 2009; Neville *et al.*, 2010). Climate warming in the Holocene resulted in retraction of montane glaciers and expansion of forests which covered about 85% of New Zealand prior to the arrival of humans (Trewick & Morgan-Richards, 2009).

New Zealand is an island of continental origin that is the emergent part of Zealandia, a sunken continent which was isolated from other parts of the Gondwanan continent for approximately 80 Myr (Trewick *et al.*, 2007). Its biota comprises both ancient lineages that are elsewhere extinct (e.g. *Sphenodon*), and organisms that are more typical of an isolated archipelago (Daugherty *et al.*, 1993). The relative contribution made to the endemic biota by vicariant versus dispersal means has long been debated (Wallis & Trewick, 2009). With the renaissance of plate tectonic theory, inter-continental distributions of organisms (including those of Australia and New Zealand), have been viewed as evidence of vicariant processes (e.g. Craw *et al.*, 1999). This approach temporarily superseded to a large extent the dispersalist paradigm of Charles



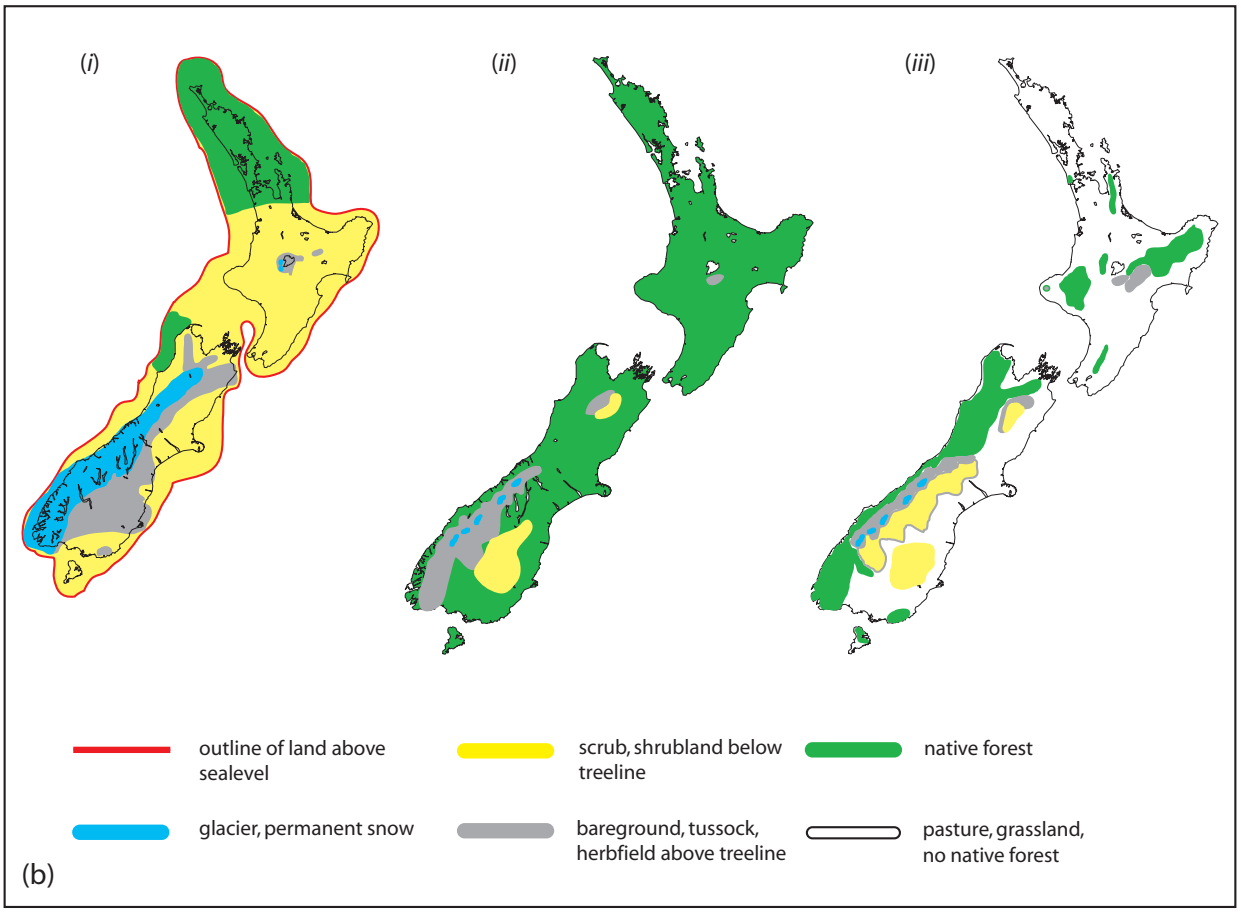


Figure 5.1: Maps of vegetation zones in Australia and New Zealand; (a) vegetation zones in Australia during LGM, modified from Hope et al., 2004, Alloway et al., 2007; (b) sequence of maps of vegetation zones in New Zealand from (*i*) LGM to (*ii*) pre human to (*iii*) modern (after human settlement) New Zealand (modified from Alloway *et al.*, 2007).

Darwin and his contemporaries. Although arguments in favour of vicariance have been made with respect to the New Zealand biota, considerable compelling evidence for dispersal has since emerged (see e.g. Goldberg *et al.*, 2008; Wallis & Trewick, 2009). The extensive marine inundation of Zealandia culminating in the late Oligocene (Cooper & Millener, 1993; Landis *et al.*, 2008) may explain the absence of many higher-level taxa in New Zealand (Daugherty *et al.*, 1993), radiation of others (Cooper & Cooper, 1995), and the close relationships of the New Zealand flora to that of Australia (Pole, 1994; Knapp *et al*., 2007).

New Zealand began to form in the Miocene and for much of its history was a relatively low-lying landmass with equitable climate (Fleming, 1979, Daugherty *et al.*, 1993, Campbell & Hutching, 2007). Pliocene orogenesis of the South Island Southern Alps (Batt *et al*., 2000, Chamberlain & Poage, 2000) was followed by Pleistocene climate cycling and glaciation (Carter, 2005). Ecological and molecular evidence indicates that plant and animal inhabitants of the (sub-) alpine zone of the Southern Alps radiated during this time (Buckley *et al.*, 2001; Lockhart *et al.*, 2001; Meudt & Simpson, 2006; Goldberg *et al.*, 2008; Wallis & Trewick, 2009). This implies that a shift from low lying habitat dominated by forests to a more diverse and heterogeneous one provided opportunities for both colonization and radiation in the Plio-Pleistocene. Substantial and rapid fluctuations in the distribution of vegetation communities during the Pleistocene (McGlone, 1985) are thought to explain the modern distribution of many cold-sensitive taxa as a result of their survival in glacial refugia (Wardle, 1963; Burrows, 1965; Dumbleton, 1969). However, the details of the phylogeographic effects of Pleistocene climate cycling on lowland taxa have been relatively little explored in New Zealand, compared to that in the Northern Hemisphere (Taberlet *et al.,* 1998; Hewitt, 1999). This is due in part to the clearing of forest by humans in New Zealand which had a major impact on its flora and fauna (figure 5.1b) (Lancashire *et al.*, 2002).

This study focuses on the grasshopper genus *Phaulacridium* Brunner v. Wattenwyl, 1893 (Orthoptera: Acrididae) that comprises five closely related species in Australia [*P. vittatum* (Sjöstedt, 1920) and *P. crassum* Key, 1992], Lord Howe Island (*P. howeanum* Key, 1992) and New Zealand [*P. marginale* (Walker, 1870) and *P. otagoense* Westerman & Ritchie, 1984] (Key, 1992). The species are relatively small (<12mm) and inhabit native and mixed exotic herb- and grasslands (Clark, 1967; Key, 1992). *Phaulacridium* are primarily lowland grasshoppers occurring up to ~1200m above sea-level in mainland New Zealand (Westerman & Ritchie, 1984) but reaching higher altitudes in equitable areas of southern New Zealand, south Australia and Tasmania (Key, 1992). Most *Phaulacridium* have non-functional reduced wings, but macropterous (fully-winged) individuals of *P. vittatum*, *P. crassum* and *P. marginale* do sporadically occur (Westerman & Ritchie, 1984; Key, 1992). In general, fewer than 3% of *P. crassum* and 10% of *P. vittatum* are macropterous (Key, 1992), although in some populations of *P. vittatum* the macropterous form is more abundant (Clark, 1967). Winged *P. marginale* are probably very rare (Bigelow, 1967). Hutton (1897) recorded two winged animals (both female) in a sample of 218 specimens. In Australia, *P. vittatum* is the most widespread

species and often becomes abundant enough to be considered a significant pest on pasture land (Australian Department of Agriculture and Food, http://www.agric.wa.gov.au/PC_92895.html?s=1385707533), whilst *P. crassum* has a very restricted range (figure 5.2a). On Lord Howe Island, approximately 575km east of Australia in the Tasman Sea, the endemic species *P. howeanum* seems to be restricted to arid rock outcrops (Key, 1992). In New Zealand, *P. marginale* is today widespread in open grasslands on the three main islands and the smaller islands in the north. It is also present on the Chatham Islands, approximately 850km east of New Zealand in the Pacific Ocean. *Phaulacridium otagoense* on the other hand has a restricted distribution, confined to arid parts of central Otago and central Canterbury in South Island, New Zealand (figure 5.2a). Both New Zealand species occupy habitat that now includes exotic vegetation.

It is generally assumed that the New Zealand *Phaulacridium* were derived from Australian lineages. Bigelow (1967) suggested that *P. marginale* (or rather its ancestor) either arrived in New Zealand during the last 10,000 years (i.e. following the start of the present interglacial), or possibly persisted in northern refugia during the Pleistocene. The justification for such a view being the scarcity in New Zealand, compared to Australia, of suitable habitat in prehuman times. Westerman and Ritchie (1984) made similar suggestions, favouring the arrival in New Zealand of the ancestor before the Pleistocene, survival of *P. marginale* in glacial refugia and the evolution of *P. otagoense in situ* during the Pleistocene. They noted as an alternative, that morphological evolution of *P. otagoense* might have been unusually rapid and extremely recent (~200y bp) and in response to human modification of habitat (Westerman & Ritchie, 1984), but conceded this to be unlikely given the extent of morphological and molecular change indicated by allozyme data. Key (1992) agreed that *P. otagoense* was probably derived from *P. marginale* during the Pleistocene, and proposed that, of the two, *P.marginale* was closest to *P. vittatum*. Westerman and Ritchie (1984) cited comparitively warm Pliocene climate as providing suitable conditions for colonization of New Zealand during that time. Although *P. marginale* habitat was likely rare during the Pleistocene, semi-arid environments like that occupied today by *P. otagoense* in central South Island were probably more widespread (figure 5.1). Thus, *P. marginale* is thought to have evolved from an Australian lineage, and *P. otagoense* to have subsequently evolved from the *P. marginale* lineage.

We apply mtDNA genealogical data to test such inferences. In particular, what are the phylogenetic relationships of the genus *Phaulacridium*, especially among the New Zealand species? What is the phylogeographic structure of New Zealand *Phaulacridium*, and does current range and abundance correlate with genetic diversity? To what extent was the colonisation and speciation history of the genus linked to Pleistocene habitat changes?

V.2 MATERIAL & METHODS

Phaulacridium grasshoppers (*P. vittatum*, *P. marginale* and *P. otagoense*) were collected by hand from lowland grasslands and stored in 95% Ethanol. DNA from fresh ethanol material was extracted from muscle tissue of one hind femur using a salting-out method (Sunnucks & Hales, 1996). Specimens from western Australia and from Lord Howe Island were previously pinned and dried; here a whole hind femur was used for DNA extraction using CTAB and phenol/chloroform extractions (Trewick, 2008). Species were identified by morphological character differences, e.g. size, sculpturing of pronotum and position of tegmina (in Australian species). Molecular analyses used primers that target the mitochondrial DNA gene cytochrome oxidase I (COI). For a subset of samples from all specimens cytochrome oxidase II (COII) and nuclear ITS and 18S genes were also amplified. In total 97 individuals, including two representative outgroup taxa (Table 5.1) were sequenced for a 763bp fragment towards the 3' end of COI using the primers C1-J2195 and L2-N-3014 (Simon *et al.*, 1994). This included 65 samples of *P. marginale* from New Zealand (including Chatham Islands), 11 *P. otagoense* from southern New Zealand, 16 *P. vittatum* from western and eastern Australia, 2 *P. crassum* from western Australia, 2 *P. howeanum* from Lord Howe Island and the outgroup (*Minyacris*) from Australia. A subset of 12 of these grasshoppers were also sequenced for a 760 bp fragment of COII using primers TL2-J-3037 and C2-N-3661 (Simon *et al.*, 1994), and representatives of 5 taxa were sequenced for a 730 bp fragment of ITS using primers ITS4 and ITS5 and a 1095 bp fragment of 18S using primers 18S-S22 and 18S-A1984 (both sets supplied by Nuclear Acid–Protein Service Unit, Biotechnology Laboratory, University of British Columbia, Vancouver). PCR reactions were performed in 10 µl volumes. The amplified products were checked on a 1% agarose gel and purified using High Pure purification columns (Roche Applied Science, Mannheim, Germany) or SAP/EXO1 digest (USB Corporation) following the manufacturer's instructions. Purified PCR products were sequenced using standard protocols for the ABI Prism BigDye Terminator Ready Reaction Kit (Applied Biosystems, Mulgrave, Australia) and run on an ABI Prism 377 automated sequencer (Applied Biosystems). Sequence identity was confirmed by comparison with published data and checked for nucleotide ambiguities in Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI, www.genecodes.com) and aligned by eye using Se-Al v2.0a11 (Rambaut, 1996).

Population analyses

The program TCS 1.21 (Clement et al., 2000) was run with a 95% connection limit to construct parsimony haplotype networks of *Phaulacridium* to assess the geographic structuring of the genus. Furthermore *P. marginale* haplotype frequencies were calculated for sampling regions in mainland New Zealand (excluding Chatham Islands). Using a prediction of spatial partitioning, mainland New Zealand was divided into three sampled zones approximately similar in spatial dimensions, using latitudinal breaks that transected the islands east to west; northern North Island (NNZ = I), central New Zealand (CNZ = II), southern South Island (SNZ = III). An additional zone was designated for the Chatham Islands (CHI = IV). These were used to test for differences

Table 5.1: *Phaulacridum* spp. sampling locations, with haplotype number, sample ID, sampling region within New Zealand corresponding to figure 5.2b (I = Northern North Island (NNZ), II = Central mainland New Zealand (CNZ), III = Southern South Island (SNZ), IV = Chatham Islands (CHI)) and coordinates (given as latitude and longitude, according to the World Geodetic System). Sample IDs stand for PH = *P. marginale,* PHo = *P. otagoense*, PHhow = *P. howeanum*, PHV = *P. vittatum*, PHcr = *P. crassum*, out = outgroup (*Minyacris*).

Haplotype	Sample ID	Location	Region	Coordinates
1.01	PH02	N.I., Taupo, Rangipo Desert	I	39º 07’ 50 / 175º 46’ 38
1.02	PH03	N.I., Wellington, Makara	II	41º 16’ 11 / 174º 42’ 22
1.03	PH04	N.I., Tongariro NP, Rangipo Desert	I	39º 17’ 26 / 175º 42’ 30
1.04	PH07	N.I., Wellington, Mt. Kaukau	II	41º 19’ 02 / 174º 40’ 04
1.05	PH09	S.I., Able Tasman NP, Awaroa	II	40º 51’ 44 / 173º 01’ 52
1.06	PH12.1	N.I., Te Urewera, Lake Waikaremoana	I	38º 45’ 19 / 177º 09’ 11
1.07	PH14	S.I., Otago, Dunedin, Kurinui Hampden	III	45º 19’ 21 / 170º 46’ 47
1.08	PH14.1	S.I., Otago, Dunedin, Kurinui Hampden	III	45º 19’ 21 / 170º 46’ 47
1.09	PH22.1	S.I., Able Tasman NP, Awaroa, Dacha	II	40º 51’ 42 / 173º 01’ 55
1.10	PH22.2	S.I., Able Tasman NP, Awaroa, Dacha	II	40º 51’ 42 / 173º 01’ 55
1.11	PH22.3	S.I., Able Tasman NP, Awaroa, Dacha	II	40º 51’ 42 / 173º 01’ 55
1.12	PH29.1	S.I., Canterbury, Hunters Hills, Myer’s Pass	III	44º 40’ 44 / 170º 43’ 18
1.13	PH29.2	S.I., Canterbury, Hunters Hills, Myer’s Pass	III	44º 40’ 44 / 170º 43’ 18
1.14	PH38	S.I., Marlborough, Clarence River	II	42º 09’ 18 / 173º 31’ 09
1.15	PH50.1	N.I., Waikato, Maungatatua	I	38º 00’ 38 / 175º 08’ 33
1.16	PH71.2	N.I., Whirinaki Forest, Rata Road	I	38º 45’ 15 / 177º 09’ 31
1.17	PHM4	S.I., Otago, Awakino	III	44º 41’ 57 / 170º 26’ 00
1.18	PHM16	N.I., East Cape, East Island	I	37º 41’ 25 / 178º 34’ 32
1.19	PHMwell	N.I., Wellington, Newlands	II	41º 13’ 33 / 174º 49’ 23
1.20	PHMco2	N.I., Coromandel	I	37º 06’ 08 / 175º 52’ 46
2.01	PH05.1	S.I., Marlborough, Pelorus Sound	II	41º 14’ 07 / 173º 46’ 51
2.02	PH06	Ch.I., Henga Scenic Res.	IV	43º 49’ 22 / 176º 22’ 39
2.03	PH06.1	Ch.I., Henga Scenic Res.	IV	43º 49’ 22 / 176º 22’ 39
2.04	PH08	Ch.I., Fakey’s Quarry	IV	43º 49’ 31 / 176º 35’ 22
2.05	PH10	Ch.I., Tuku Res.	IV	44º 03’ 26 / 176º 38’ 47
2.06	PH11	Ch.I., Matarakau	IV	44º 03’ 25 / 176º 38’ 47
2.07	PH18.1	Ch.I., Tuku Res., Trapline	IV	44º 03’ 26 / 176º 38’ 47
2.08	PH19.1	Ch.I., Tuku Res., Abyssinia Track	IV	44º 03’ 27 / 176º 38’ 47

2.09	PH19.2	Ch.I., Tuku Res., Abyssinia Track	IV	44º 03’ 27 / 176º 38’ 47
2.10	PH23.1	S.I., Lewis Pass NP, Marble Hill Picnic Area	II	42º 21’ 13 / 172º 12’ 34
2.11	PH24	S.I., Canterbury, Hunters Hills, Mackenzie Pass	III	44º 11’ 37 / 170º 34’ 44
2.12	PH27.1	S.I., Mt. Cook NP, Mt. Cook Village	III	43º 42’ 30 / 170º 44’ 20
2.13	PH27.2	S.I., Mt. Cook NP, Mt. Cook Village	III	43º 42’ 30 / 170º 44’ 20
2.14	PH27.3	S.I., Mt. Cook NP, Mt. Cook Village	III	43º 42’ 30 / 170º 44’ 20
2.15	PH28.1	S.I., Canterbury, Hunters Hills, Myer’s Pass	III	44º 40’ 44 / 170º 43’ 18
2.16	PH32.1	S.I., Otago, Queenstown, Coronet Peak Skifield	III	44º 55’ 33 / 168º 44’ 07
2.17	PH36	S.I., Canterbury, Arthurs Pass, Waimakariri River Valley	III	43º 01’ 52 / 171º 40’ 56
2.18	PH37	S.I., Seaward Kaikoura Range, Mt. Fyffe	II	42º 18’ 29 / 173º 37’ 02
2.19	PH39.1	S.I., Marlborough, Kekerengu, Dee Stream	II	41º 59’ 56 / 173º 44’ 46
2.20	PH71.1	N.I., Whirinaki Forest, Rata Road	I	38º 45’ 15 / 177º 09’ 31
2.21	PHM1	S.I., Marlborough, Mt. Patriarch	II	41º 36’ 35 / 173º 13’ 17
2.22	PHM2	S.I., Nelson, St. Arnaud, Mt. Roberts	II	41º 50’ 51 / 172º 47’ 55
2.23	PHM3	S.I., Seaward Kaikoura Range, Mt. Fyffe	II	42º 18’ 29 / 173º 37’ 02
2.24	PHM7	S.I., Otago, Awakino	III	44º 41’ 57 / 170º 26’ 00
2.25	PHM10	S.I., Otago, St. Bathans	III	44º 52’ 16 / 169º 48’ 48
2.26	PHM13	S.I., Canterbury, Old Man Range	III	43º 58’ 49 / 170º 23’ 06
2.27	PHMco3	N.I., Coromandel	I	37º 06’ 08 / 175º 52’ 46
2.28	PHBurk4	S.I., Canterbury, Lake Tekapo, Burks Pass	III	44º 05’ 29 / 170º 36’ 06
3.01	PH13	Ch.I., Awatotora	IV	44º 01 48 / 176º 39’ 23
3.02	PH31.1	S.I., Fiordland, Borland Lodge	III	45º 46’ 28 / 167º 32’ 10
3.03	PH35.1	S.I., Canterbury, Cave Stream Scenic Res.	III	43º 11’ 51 / 171º 44’ 39
4.01	PH17.1	Ch.I., Maipito Road	IV	43º 57’ 57 / 176º 33’ 01
4.02	PH26	S.I., Mt Cook NP, Mt Cook Village	III	43º 42’ 30 / 170º 44’ 20
4.03	PH30.1	N.I., Lake Taupo, Kinloch, Kawakawa Track	I	38º 40’ 09 / 175º 55’ 40
4.04	PH49	N.I., BOP, Te Puke	I	37º 46’ 51 / 176º 20’ 21
5	PH25	S.I., Mt Cook NP, Mt Cook Village	III	43º 44’ 25 / 170º 43’ 18
6	PH52	N.I., Manawatu, Levin	II	40º 41’ 05 / 175º 08’ 52
7	PH54	N.I., Hawkes Bay, SH5	I	39º 44’ 30 / 177º 00’ 22
8.01	PH55.2	N.I., Coromandel, Whitianga	I	36º 53’ 05 / 175º 49’ 12

8.02	PH55.3	N.I., Coromandel, Whitianga	I	36º 53’ 05 / 175º 49’ 12
9	PH57.2	S.I., Canterbury, Lake Tekapo, Burkes Pass	III	44º 05’ 29 / 170º 36’ 06
10	PH71.3	N.I., Whirinaki Forest	I	38º 45’ 15 / 177º 09’ 31
11.01	PM5	S.I., Marlborough, Mt Lyford	II	42º 28’ 02 / 173º 08’ 25
11.02	PHM6	S.I., Otago, Awakino	III	44º 41’ 57 / 170º 26’ 00
12	PHM26	N.I., Great Barrier Is, Copper Mine	I	36º 15’ 03 / 175º 24’ 06
13.01	PHoM11	S.I., Otago, Alexandra, Graveyard Gully		45º 15’ 52 / 169º 23’ 45
13.02	PHoM12	S.I., Otago, Alexandra, Graveyard Gully		45º 15’ 52 / 169º 23’ 45
14	PHoM14	S.I., Otago, Alexandra, Manor Burn		45º 21’ 12 / 169º 36’ 48
15.01	PHoM8	S.I., Otago, Alexandra, Conroys Dam		45º 17’ 08 / 169º 18’ 47
15.02	PHoCON1	S.I., Otago, Alexandra, Conroys Dam		45º 16’ 46 / 169º 19’ 28
16	PHoM15	S.I., Otago, Alexandra, Bridge Hill		45º 15’ 32 / 169º 22’ 36
17	PHoM15.1	S.I., Otago, Alexandra, Bridge Hill		45º 15’ 32 / 169º 22’ 36
18	PHo59	S.I., Mackenzie, Benmore Range, Twizel		44º 19’ 57 / 170º 04’ 03
19	PHo60	S.I., Mackenzie, Simons Pass Hill, Tekapo River		44º 12’ 07 / 170º 18’ 26
20	PHo51	S.I., Mackenzie, Lake Tekapo		44º 10’ 55 / 170º 34’ 38
21	PHoMtJohn	S.I., Mackenzie, Lake Tekapo, Mt John		45º 43’ 01 / 170º 23’ 11
22	PHhow46	Lord Howe Is., Mt Gower, Big Hill Saddle		31º 34’ 59 / 159º 04’ 58
23	PHhow47	Lord Howe Is., Mt Gower, Big Hill Saddle		31º 34’ 59 / 159º 04’ 58
24.01	PHV1	Australia, Victoria, La Trobe		37º 43’ 35 / 145º 02’ 48
24.02	PHV3	Australia, Victoria, Ngambie		36º 47’ 15 / 145º 08’ 54
24.03	PHV4	Australia, Victoria, La Trobe		37º 43’ 35 / 145º 02’ 48
24.04	PHV79.1	Australia, Tasmania, The Springs		42º 42’ 46 / 146º 59’ 31
24.05	PHV81	Australia, Tasmania, Kempton, Elderslie		42º 35’ 32 / 147º 06’ 38
24.06	PHV83.1	Australia, Tasmania, Bothwell, Interlaken		42° 08’ 50 / 147° 10’ 37
25	PHV2	Australia, Victoria, Ballarat Airport		37º 31’ 12 / 143º 46’53
26	PHV5	Australia, NSW, Rules Point		34º 47’ 36 / 148º 27’ 17
27	PHV80	Australia, Tasmania, Upper Scamander		41º 26’ 54 / 148º 13’ 16
28.01	PHV40.2	Australia, WA, Frankland		34º 21’ 45 / 117º 04’ 56
28.02	PHV44.1	Australia, WA, Esperance		33º 45’ 21 / 121º 45’ 07
29	PHV43.1	Australia, WA, Manjimup		34º 14’ 59 / 116º 09’ 31

30	PHV43.2	Australia, WA, Manjimup	34º 14' 59 / 116º 09' 31
31.01	PHV44.2	Australia, WA, Esperance	33º 45' 21 / 121º 45' 07
31.02	PHV40.1	Australia, WA, Frankland	34º 21' 45 / 117º 04' 56
32.01	PHcr68	Australia, WA, Lancelin	31º 02' 07 / 115º 20' 32
32.02	PHcr75	Australia, WA, Lancelin	31º 02' 07 / 115º 20' 32
out		*Minyacris nana*	
out		*Minyacris occidentalis*	

in genetic composition spanning New Zealand (figure 5.2b). Under panmixis it is expected that no significant partitioning of genetic diversity would exist, however, any reduction in gene flow is expected to result in genetic diversity (haplotype variation and frequency) being partitioned in space. The program DnaSP v5.0 (Rozas *et al.*, 2003) was used to calculate haplotype diversity (*h*), nucleotide diversity (π, Nei, 1987) and the average number of nucleotide differences (*k*) within *P. marginale* populations and the two distinct populations of *P. otagoense*. Additionally, Tajima's *D* statistic (Tajima, 1989) was calculated for the different regions as it provides a useful indicator for neutral markers such as the mitochondrial DNA of population range expansion and exchange (Ray *et al.*, 2003; Wegmann *et al.*, 2006). Furthermore mismatch distributions for the mainland New Zealand species *P. marginale* and *P. otagoense* were calculated and a Mantel-test for isolation by distance (IBD) was run for mainland *P. marginale* samples using IBDWSv.2 (Jensen *et al.*, 2005).

Phylogenetic Analysis

Distance estimation and phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford, 1998). We conducted neighbor-joining (NJ) and Maximum Likelihood (ML) analyses, as implemented in PAUP* with the entire COI dataset and with a subset of 31 samples respectively. We used 1000 bootstrap replicates to test the tree topology under ML and NJ criteria.

In addition we employed Mr.Bayes 3.1.2 (Ronquist & Huelsenbeck, 2003) to examine tree topology with a COI – COII dataset under a six parameter model similar to that selected for ML analysis by Modeltest 3.7 (Posada & Crandall, 1998). We used the GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites. The same model was applied to the two partitions (COI and COII) with rates and nucleotide frequencies for each gene unlinked. We used four independent MCMC runs for ten million generations with a burn in of 25%. Resulting posterior probabilities on the nodes were recorded.

Additionally we used two nuclear genes (ITS and 18S) to help resolve the phylogenetic relationships within this genus and run analyses in Paup* with a subset of 5 species from New Zealand and Australia.

V.3 RESULTS

Population

Statistical parsimony networks were generated in TCS containing all COI sequences of all *Phaulacridium* species (figure 5.2a). In a sample of 65 specimens of *P. marginale* from mainland New Zealand we found 12 different COI haplotypes (figure 5.2a) with uncorrected genetic distances up to 0.018. Two dominant haplotypes were found with 20 and 28 specimens each, distributed throughout New Zealand (figure 5.2b, inset (*i*)). Additionally several singletons were encountered in all sampling regions except the Chatham Islands. Strikingly, in contrast to the situation in *P. marginale*, *P. otagoense* diversity splits into two separate haplotype networks, each representing one of the two small areas in southern New Zealand where this species is found (figure 5.2a). This species displays a higher genetic diversity than the widespread *P. marginale* with nine haplotypes found in 11 grasshoppers. The calculated uncorrected pairwise genetic distances within this species ranged from 0 – 0.015 in the Alexandra population (NZ*ota*Alex) and 0.001 – 0.018 in the more northern Mackenzie population (NZ*ota*Mack) (figure 5.2a). The maximal genetic distance between the two *P. otagoense* populations was 0.037 (distance between these locations is approx. 180 km), approximately as high as the genetic distance to *P. marginale* with up to 0.035. Mismatch distributions calculated for *P. marginale* and *P. otagoense* highlight the difference in genetic divergence within these two species (figure 5.3a).

Phaulacridium vittatum from Australia also split into two haplotype networks, one displaying haplotypes from eastern Australia with addition of the Lord Howe Island species (EA*how*) and the other grouping the western Australian *P. vittatum* with the western Australian *P. crassum* (WA*cras*) (figure 5.2a). Genetic distances within the Australian haplotype networks were up to 0.006 and 0.013 respectively. The western Australian grasshoppers show genetic distance of up to 0.044 to eastern Australian samples, 0.05 to *P. marginale* from New Zealand and 0.055 to *P. otagoense*.

Populations of *P. marginale* displayed very low levels of DNA nucleotide diversity (π) within mainland New Zealand and the Chatham Islands (Table 5.2). The sequences of the northern area had the highest level of haplotype and nucleotide diversity of all sampling regions. The IBD plot calculated for mainland New Zealand *P. marginale* (excluding Chatham Islands) showed that there was no correlation between genetic and geographic distances in these samples (figure5.3b). Tajima *D* tests (Tajima, 1989) were only significant for the SNZ dataset and the total population (Table 5.2). Negative Tajima's *D* statistics for the different populations indicate an excess of sites with low frequency polymorphisms, i.e. the population has yet to reach equilibrium (Tajima, 1989). The two *P. otagoense* populations, not surprisingly, showed high levels of π compared to *P. marginale* (Table 5.2).

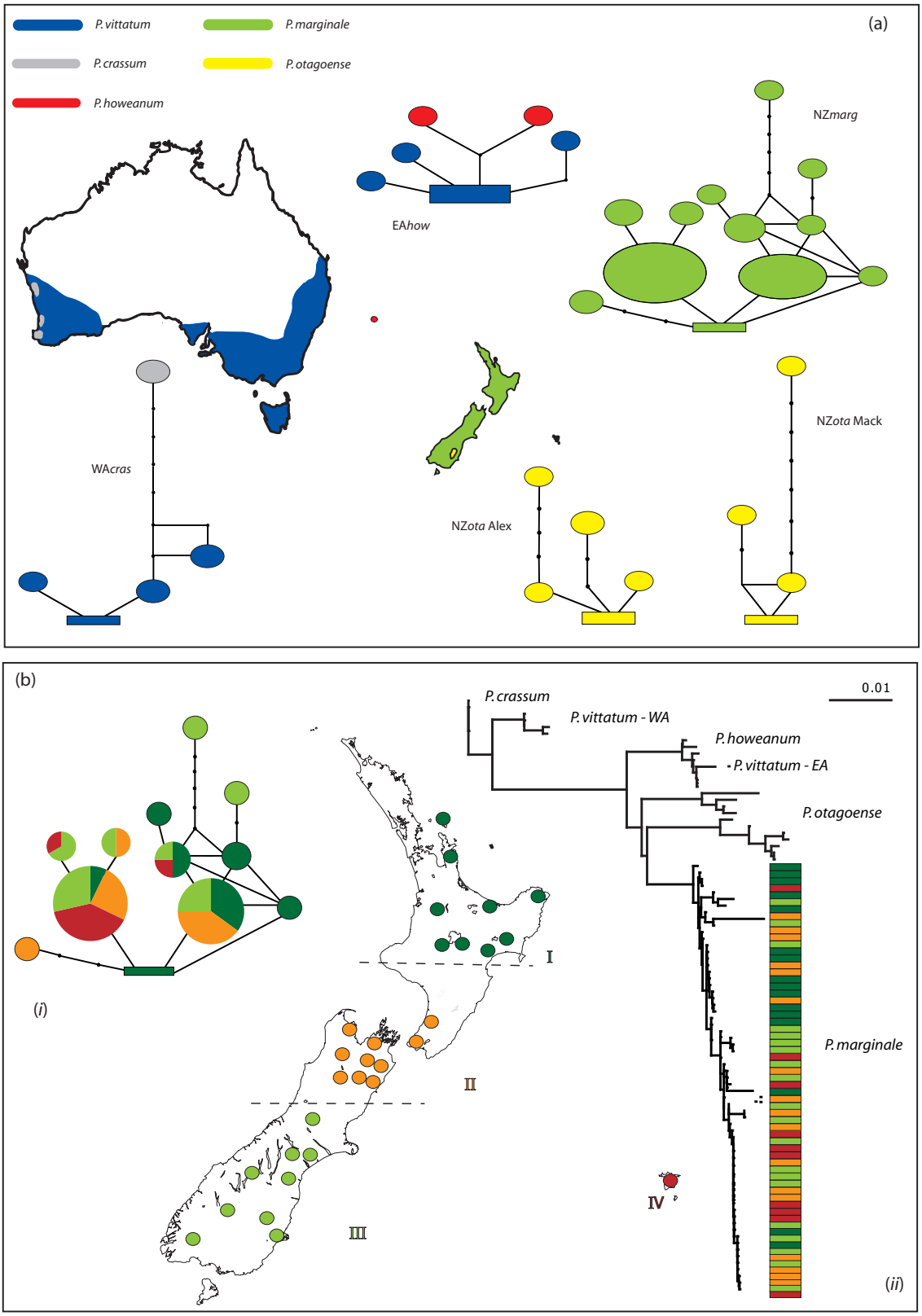


Figure 5.2: Maps of Australia and New Zealand showing distribution and haplotype networks of taxa. (a) Australian and New Zealand region with distribution of *Phaulacridium* taxa coloured and matching circles in corresponding haplotype networks (NZ*marg* = New Zealand *P. marginale*, NZ*ota* Alex = New Zealand *P. otagoense* from Alexandra area, NZ*ota* Mack = New Zealand *P. otagoense* from Mackenzie area, EA*how* = P. vittatum eastern Australia and *P. howeanum*, WA*cras* = *P. vittatum* western Australia and *P. crassum*). (b) Map of New Zealand showing the four main sampling areas (I – IV, three in mainland New Zealand and the fourth being the Chatham Islands) of *P. marginale* with sampling location (coloured circles). Inset (*i*) shows a haplotype network for *P. marginale*, with colours corresponding to sampling areas and depicting the frequency of the different areas within one haplotype; inset (*ii*) shows a Neighbour-Joining tree representing all sampled taxa, with coloured labels of *P. marginale* representing haplotypes according to sampling areas and coloured circles in map.

Table 5.2: DNA variation and haplotype diversity within and between regional samples of *Phaulacridium marginale* in the New Zealand region and the two populations of *Phaulacridium otagoense*, with the sample size for each region (n), number of observed haplotypes (N_{haps}), average number of nucleotide differences (*k*), nucleotide diversity (π x10^{-3}), number of polymorphic sites (*S*), Tajima's *D* (*P<0.05) and haplotype diversity (*h*). Region abbreviations correspond to abbreviations in Table 5.1 and figures 5.2a & b (NNZ = Northern North Island (I), CNZ = Central Mainland New Zealand (II), SNZ = Southern South Island (III), CHI = Chatham Islands (IV)); NZ*ota* Alex = *P. otagoense* population in Alexandra, Otago; NZ*ota* Mack = *P. otagoense* population in Mackenzie, Canterbury.

Area	n	N_{haps}	*k*	π	*S*	Tajima's *D*	*h*
NNZ	16	3	0.358	0.81	2	-0.3817	0.342
CNZ	17	2	0.353	0.80	3	-1.706	0.118
SNZ	22	3	0.273	0.62	3	-2.140*	0.177
CHI	10	1	0	0	0	-1.401	0
Total population	65	5	0.274	0.62	7	-2.051*	0.180
CHI/NNZ			0.225	0.45			
CHI/CNZ			0.296	0.65			
CHI/SNZ			0.438	0.82			
NNZ/CNZ			0.360	0.80			
CNZ/SNZ			0.308	0.70			
NNZ/SNZ			0.360	0.75			
NZ*ota* Alex	7	3	2.095	3.50	6	-0.863	0.905
NZ*ota* Mack	4	5	4.667	7.79	9	-0.491	0.833
Total population	11	8	9.800	16.36	23	0.680	0.945

Phylogenetic Analysis

The two nuclear genes (ITS and 18S) displayed little variation among species. ITS showed 3 variable sites out of 730bp and in 18S there was no variation within 1095bp of sequence and it was therefore not feasible to run phylogenetic analyses with these markers. However these data (not shown) is consistent with the shallow evolutionary history of the genus indicated by mitochondrial DNA data.

Neighbour-Joining phylogenetic analysis of the whole COI dataset resulted in a resolved haplotype tree (figure 5.2b inset (*ii*)) illustrating the closer relationship of the western Australian *P. vittatum* specimens to individuals of *P. crassum* compared to other populations of the same

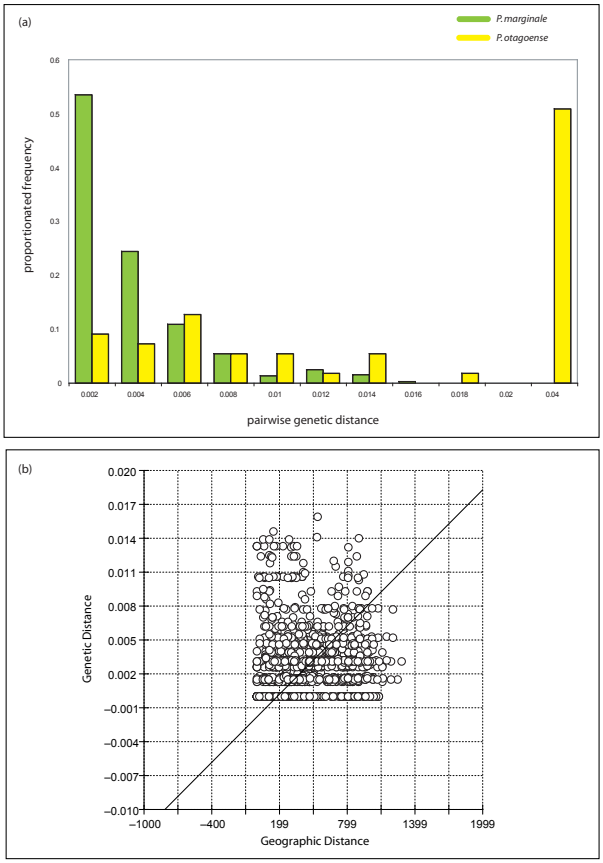


Figure 5.3:(a) Mismatch distribution plot calculated with proportionated frequencies and pairwise genetic distances of *P. otagoense* and the mainland population of *P. marginale*.
(b) Result of a Mantel-test for isolation by distance (IBD) run for mainland *P. marginale* samples using IBDWSv.2 (Jensen *et al.*, 2005).

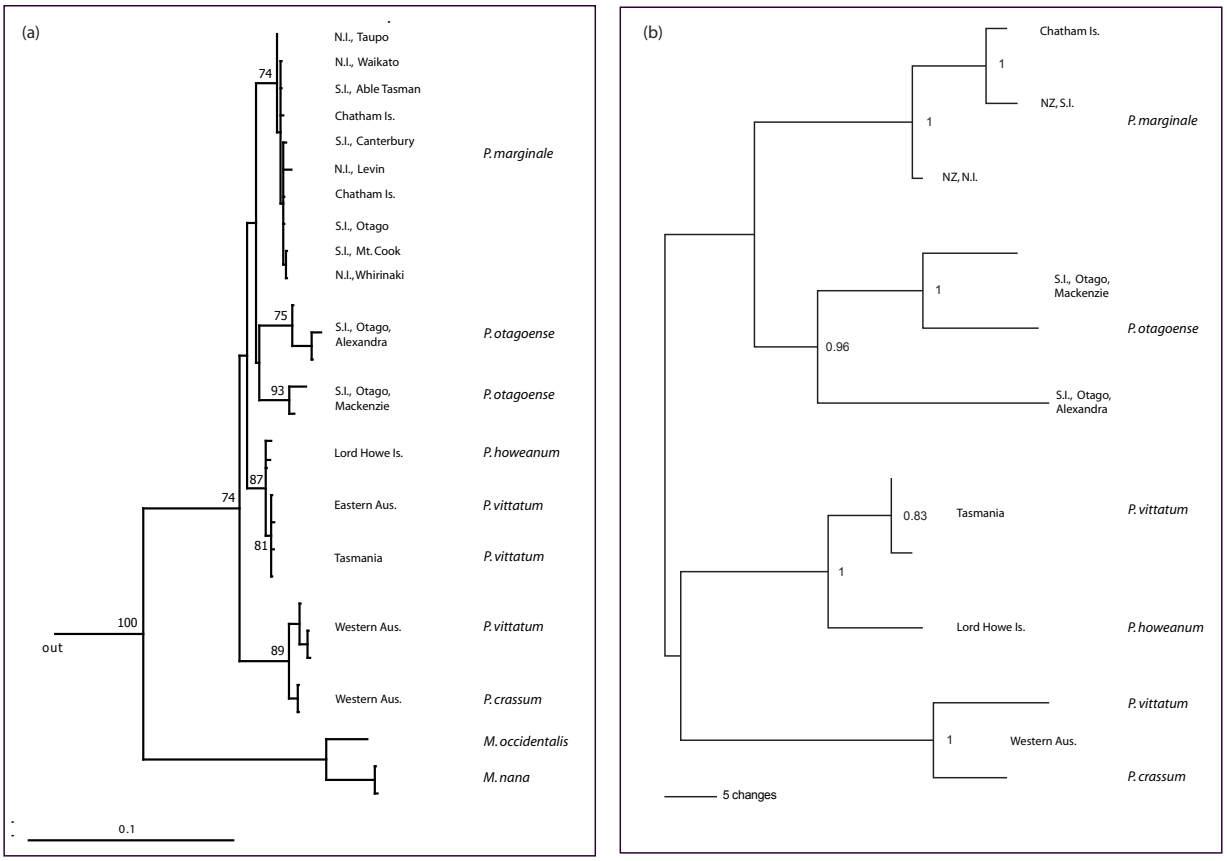


Figure 5.4: (a) COI ML tree of a 31 taxa subset of all *Phaulacridium* species used in this study, numbers on branches show bootstrap support for the nodes (1000 replicates); the tree was rooted with the outgroup taxa *Minyacris nana* and *Minyacris occidentalis* from Australia; (b) Tree of a 11 taxa subset of all *Phaulacridium* species used in this study for COI-COII generated in MrBayes with values for Bayesian posterior probabilities mapped at the branches.

species, as well as closer affinity of the *P. howeanum* to the eastern Australian samples of *P. vittatum*. Maximum Likelihood (ML) analysis of COI generated an evolutionary hypothesis for a subset of 31 grasshoppers (representing the five *Phaulacridium* species plus the outgroup) (figure 5.4a), displaying the same phylogenetic relationships as shown in figure 5.2b (inset (*ii*)). Bootstrap support (1000 replicates) was not high for the main nodes, a common problem encountered when species are closely related. However, *P. crassum* resolved as sister to western Australian *P. vittatum* (Bootstrap = 89) and *P. howeanum* as sister to eastern Australian *P. vittatum* (Bootstrap = 87).

The Bayesian analysis of COI and COII for a set of 11 specimens covering the whole taxonomic and geographic range of the genus returned one tree (this tree was midpoint rooted) with strong support for the internal nodes but it failed to resolve the relationship between New Zealand and Australian species (figure 5.4b).

V.4 DISCUSSION

Phaulacridium is an interesting example of a trans-Tasman distribution in animals as there are relatively few instances where species of the same genus are distributed in Australia and New Zealand. Although these grasshoppers are mostly not fully winged this does not appear to prevented dispersal across wide expanses of land and ocean. The geographic range of *Phaulacridium* is large by southern hemisphere standards as it extends approximately 4000km from west to east Australia, and a further 1500km across the Tasman Sea to New Zealand and the Chatham Islands. Within this range, five species are recognised, but the individual ranges of these differ enormously. Three species have relatively small geographic ranges, *P. crassum* near the coast in Western Australia, *P. howeanum* on a rock outcrop on Lord Howe Island (Tasman Sea) and *P. otagoense* in semi-arid land in central South Island, New Zealand (figure 5.2a). The other two species are more widespread but comprise three disjunct geographic populations, *P. vittatum* west and east of the Nullarbor Plain in southern Australia, and *P. marginale* in New Zealand. Eastern *P. vitattum* also extend to Tasmania (figure 5.2a), and *P. marginale* to the Chatham Islands (figures 5.2a & b). These species distributions include two cases of parapatric species pairs (*P. crassum* surrounded by *P. vittatum* in Western Australia and *P. otagoense* by *P. marginale* in southern New Zealand) and suggest speciation may have involved adaptation to local microclimate.

Phylogeographic data add to this picture, revealing that in Australia neighbouring populations are more closely related to one another than geographically more distant populations, even to the extent that western *P. vittatum* are sister to parapatric *P. crassum*, and eastern *P. vittatum*

are sister to *P. howeanum*. Consequently *P. vittatum* is paraphyletic with respect to these two species. However, the situation in New Zealand is rather different, as the two local endemics are, by comparison, more deeply diverged from one another. There is higher genetic diversity in the locally restricted *P. otagoense*, compared to the widespread *P. marginale*, and support for monophyly of the New Zealand taxa is rather weak. Thus the mitochondrial data fails to confirm the hypothesis that *P. otagoense* is derived from *P marginale* (Key, 1992; Westerman & Ritchie, 1984). While *P. marginale* is widespread today, population genetic analyses suggest this is the result of recent population expansion. Genetic diversity in this species is low, even when New Zealand and Chatham Islands samples are compared. On the other hand, high genetic diversity in *P. otagoense*, despite a small modern range, suggests that this species had a comparatively larger population size and range in the recent past. A comparatively larger historic population is the simplest explanation for the retention of high genetic diversity in this now local species. Support for this idea comes from the observation that the habitat/region occupied by *P. otagoense* today probably represents the most extensive natural lowland grass/scrubland available in New Zealand prior to human colonisation. Other extensive native grasslands are those above the treeline, but these are occupied by "alpine" grasshoppers (*Sigaus*, *Brachaspsis* and *Alpinacris*; Bigelow, 1967). This pattern emphasises the need to consider past climatic and geological events when interpreting extant patterns of diversity (Graham *et al.*, 2006).

Vegetation patterns inferred for the Pleistocene LGM in New Zealand indicate that during glacials, cooling and drying in the east led to the development of extensive grasslands/ shrublands (McGlone *et al.*, 1993). An increase in grassland habitat in concert with a westerly airflow (Sanmartin *et al.,* 2007) may have improved the chances of LDDE (long distance dispersal and establishment) of New Zealand by *Phaulacridium*. The near entire coverage by forest of non-alpine New Zealand during interglacials would, conversely, have severely limited habitat availability for *Phaulacridium*. Consequently, it is likely that *P. marginale* range was most limited during interglacial episodes rather than glacials (in contrast to at least one alpine orthopteran; Trewick *et al.*, 2000). Suitable natural habitat was probably limited to grassland patches associated with disturbed environments around rivers and frost flats. Even by 1840, it is estimated that lowland swards covered no more than 2% of mainland New Zealand (Mark & McLennan, 2004). The pattern of the widespread New Zealand species *Phaulacridium marginale* does not reveal specific locations of refugia, as has been observed in Northern Hemisphere glaciated regions, but shows the typical pattern of recent range expansion, with low genetic diversity. The fact that, until the arrival of humans in New Zealand (~1000 years ago), the only "substantial area below tree line without complete forest cover was central Otago" (McGlone *et al.*, 1993) suggests that this semi-arid area (e.g. Walker *et al.*, 1995) or "rain-shadow grasslands" (McGlone, 2001) may have been a refuge for *P. otagoense* during interglacials. Because this area is influenced by its distance from oceans and adjacent mountain ranges, grassland habitat could have existed here since formation of the Southern Alps in the Pliocene (i.e. before Pleistocene global climate cooling).

Forest clearance by humans in New Zealand resulted in the expansion of native and subsequently mixed exotic grassland habitat available for *P. marginale* to expand its range. By the early 1800's it is estimated that grassland covered ~30% of mainland New Zealand (Mark & McLennan, 2004), and the process contintues today (Scott, 1979). The two distinct populations of *P. vittatum* in eastern and western Australia are today separated by the Nullarbor Plain, a large semi-arid area that stretches approximately 1,200km from Western Australia to southern Australia (Key, 1992). Many other grasshoppers occupy this region including plague forming species of *Austroicetes*. Mitochondrial lineages of *P. vittattum* are distinctive to west and east Australia and lack monophyly and are likely to have been separated at least since the LGM as vast areas of southern and Western Australia, including the area that is now the Nullarbor, supported arid desert habitat (Hope *et al.*, 2004). We find two morphologically distinct species (*P. howeanum* and *P. crassum*) differ from the respective *P. vittatum* populations by small mitochondrial DNA distance, that renders them within the respective mitochondrial clade of *P. vittatum* regional populations.

The New Zealand lineages might be sister to the eastern Australian lineages as suggested by NJ and ML analyses with an outgroup (figures 5.2b(*ii)*; 5.4a & b), which is indicative of patterns of west to east expansion of the genus. However, the number and direction of trans-Tasman dispersal events cannot be confirmed with the current dataset, so we cannot exclude colonization hypotheses previously erected (Westerman & Ritchie, 1984; Key, 1992). However, shallow mitochondrial DNA genetic distance between New Zealand and Chatham Island populations and lack of unique Chatham haplotypes suggest that these islands have been recently colonized by *P. marginale* from mainland New Zealand.

The population composition of the New Zealand species is quite unexpected, with two lineages of P. *otagoense* apparently occupying distinct areas and retaining high genetic diversity (π =16.36) in a relatively small space, compared to a very widespread lineage (*P. marginale*) with relatively low genetic diversity (π =0.62) (figures 5.2 & 5.3a). The genetic diversity within the two *P. otagoense* populations is more or less as high as the diversity in the population of *P. marginale* (Table 5.2). In fact the genetic diversity is so high and the phylogenetic signal deeply structured that the possibility arises that the two distinct *P. otagoense* lineages from Alexandra and Mackenzie are actually two biological entities.

Phaulacridium examplifies the manner in which phylogeography can be driven by multiple factors on different time scales. The most important factors in geologically recent times appear to have been the uplift of the Southern Alps in the Pliocene (5 Ma) creating new alpine habitat and rain-shadow habitat, and Pleistocene (2.4 Ma) climate cooling events restricting available habitat in New Zealand. Both factors likely favoured species that are more adapted to dry and cold environment. This is still apparent in southern New Zealand nowadays, that has a well developed and diverse alpine adapted biota that exhibits much higher interpopulation genetic diversity than many species more adapted to warmer climate zones. In Australia, Pleistocene climate cycling had similar effects on the biota on a larger geographic scale, with locally

distributed high haplotype diversity within populations in many taxa (Byrne, 2008), resulting in divergent lineages. This structure can also be found in the present sampling of Australian *Phaulacridium* with deeply divergent lineages within populations.

When compared to northern hemisphere grasshoppers, there are stark contrasts at the population and species level depending on habitat zones occupied. In Europe low genetic diversity in the lowland meadow grasshopper (Lunt *et al.*, 1998) indicate widespread extirpation in the LGM, followed by extensive range expansion. Montane grasshoppers (e.g. *Melanoplus*) of North America however, are species-rich and highly structured among mountain ranges (Knowles, 2000). Diversity within *Phaulacridium* is more like that of northern lowland species, but intriguingly this pattern of range expansion and speciation has operated across a region with widely spaced habitat patches.

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VI

Exploring biogeographic congruence in a continental island system

ABSTRACT

In phylogeographic studies it is generally expected to find similar patterns of lineage diversity and timing within the same landscape under the assumption that these lineages have responded to past environmental changes (biotic and abiotic) in comparable ways. This is to some extent recognisable in many cases in continental biotic assemblages and even on oceanic island systems, like Hawaii. To examine whether congruence is evident among lineages within New Zealand, regarding their response to past geophysical events, data for taxa represented in mainland New Zealand and the Chatham Islands were gathered. Five invertebrate taxa were included in this study from three different orders, all flightless. One taxon was an endemic forest-dwelling genus of Raphidophoridae (Orthoptera: *Talitropsis*) that has one widespread species in New Zealand and two endemic species on the Chatham Islands. Additionally three genera of the order Coleoptera were investigate, including stag beetles, weevils and clickbeetles. Furthermore the widespread earwig *Anisolabis* was included, that is common on beaches in New Zealand and the Chathams. This was the only taxon included not having an endemic species on the Chatham Islands. Mitochondrial DNA was employed to reconstruct phylogenetic relationships within the lineages and population genetic information was gathered to compare for consistency within the sampling. The findings corroborated other molecular studies on the biota of New Zealand. There was no obvious congruent pattern between the sampled taxa and so far there is no uniform relation obvious between lineage formation, landscape history and distribution of taxa.

VI.1 INTRODUCTION

A simple prediction in biogeography is that different taxon groups will show similar patterns of distribution and phylogeny if their evolution has been in response to the same historic processes. Some biogeographers have used such a proposal as the basis of a putative test of the role of vicariance in biogeography under the assumption that patterns associated with dispersal would by their nature not be coincident (Rosen, 1978; Zink *et al.*, 2000; Ebach *et al.*, 2003). However, early in the application of phylogeographic information using molecular data it was predicted that dispersal could be expected to yield congruent biogeographic patterns, where the taxa involved were responding to a common cause (e.g. Taberlet *et al.*, 1998) and in many contexts the perceived distinction between vicariance and dispersal processes in biogeography is illusional (Crisp *et al.*, 2011). In continental systems the past location of species' refugia has been identified using phylogeographic data and found to coincide for many taxa, as does the general trend of expansion from refugia (Avise, 2000; Hewitt, 2004). On oceanic islands a general trend in the phylogeographic history of taxa colonising successive islands as they emerged, has been encountered (Fleischer *et al.*, 1998). However, discordance is also found and whilst this might be attributed to an overwhelming influence of stochastic events, it might also reflect differences, at least to some extent, in the ecology of the organism concerned. Obvious features that might make a difference would be those associated with mobility, population size and reproductive strategy that might influence establishment success (Carlquist, 1974). Distinguishing between random effects and those linked to species traits is very difficult, especially because traits associated with dispersal might be selected against after colonisation (Carlquist, 1974; Vittoz & Engler, 2007).

The study of New Zealand biogeography has in the past focused on the role of plate tectonic vicariance in the origins of New Zealand lineages, but more recently molecular data have shown that many lineages have arrived relatively recently and that diversification is young in many taxon groups. Furthermore, it is increasingly evident that there is no uniform pattern in the phylogeographic structuring of the biota within New Zealand (Goldberg *et al.*, 2008; Wallis & Trewick, 2009), with so far, no consistent linkage between lineage formation, landscape history or distribution of taxa evident. Although New Zealand was influenced by Pleistocene climate cycling, this does not by itself explain the phylogeographic structure of taxa we see today because not every lineage in New Zealand is younger than the last glacial maximum (LGM) (Goldberg *et al.*, 2008; Wallis & Trewick, 2009), yet it is obvious that every species and population must have been affected by climate cycling. Although some similarity can be found in lineage age for different taxa, the pattern of spatial structuring is not consistent among lineages. This was a plausible expectation of species that have similar ecological requirements (e.g. habitat, microclimate, diet) and have experienced range retraction and expansion in a similar time frame (e.g. since the LGM). Instead what we tend to observe among the extant biota in New Zealand

is that some endemic taxa have retained a relatively high genetic and taxonomic diversity, but are usually quite rare and threatened by extinction and often persist only in protected areas such as on offshore predator controlled islands (e.g. many beetles taxa, weta etc.). On the other hand there are endemic taxa that are widespread with low genetic diversity and in most cases comprising a single mainland New Zealand species (e.g. woodpigeon - chapter IV, and see Trewick & Gibb 2010) or a combination of both with widespread species showing low genetic diversity and localized populations/species that retained high genetic diversity within small ranges (e.g. *Phaulacridium* grasshopper - chapter V; weta, Trewick & Morgan-Richards, 2005). It is therefore valuable to sample taxa across a variety of different ecological and evolutionary backgrounds and compare their genetic, taxonomic and spatial structuring if we are to develop a better picture of the biotic assemblages of present day New Zealand biota and its evolutionary response to biotic and abiotic factors that influenced it in the past.

The Chatham Islands

The Chatham Islands are located on the Pacific Plate (S 44°03'47.16" and W 175°57'35.73") approximately 850 km east of mainland New Zealand (figure 6.1). The islands are situated on the eastern end of the Chatham Rise, a submerged ridge-structure extending from mid-South Island of New Zealand to the Chatham Islands. The ridge, together with the Chathams, is part of the same continental crust on which New Zealand is located, and only 10% of the Chathams landmass is above sea level nowadays (Stevens, 1980). The present land above sea level consists of two inhabited main islands (Main and Pitt Island) and several smaller islets and rocks (figure 6.1). Paleomagnetic studies provide evidence that the position of the islands on the eastern tip of the Chatham Rise has been more or less fixed since the break up of Gondwanaland (Campbell *et al.*, 1993). This finding presumably led to the hypothesis that the Chathams have been stable since then (80 Ma) and therefore harbour ancient biota (Craw, 1988). No geological evidence has been found so far that the rise has ever been emergent to provide a land bridge between New Zealand and the Chatham Islands. The geology of the Chathams is in some way special compared to most of the other small Pacific island groups because their basement rocks consist of old metamorphic rocks (Chatham schist) which is very similar to the old schist rocks known from the foot of the Southern Alps of Otago and Canterbury. Today the Chatham schist is only exposed in the northern part of the Chathams and on the Forty Fours and its metamorphosis is dated to 160 Myr. On top of this old layer are several younger deposits from the Cretaceous and Cenozoic which contain the oldest fossils on the Chathams. These fossils indicate a sustained isolation of the Chatham area for the last 65 Myr (Campbell *et al.*, 1993). In contrast to its settled behaviour today, the Chatham area has a long history of volcanic activity, mainly between 60-40 Ma (Eocene to Oligocene) and 5-1.6 Ma (Pliocene). In the latter period most of the smaller islands were formed.

Although in the past it was assumed that the Chatham Islands had existed as land since the the break-up of Gondwanaland, it is likely that islands in this area have been submerged and emerged several times in response to regional tectonic uplift events that affected the entire

eastern end of the Chatham Rise. The last emergence event was most probably no more than 4 Ma (million years ago) (Late Pliocene) (Campbell, 1998; Campbell *et al.*, 2006). Geological studies have shown that phases of climate cooling in the Pleistocene (2-1.5 Ma) influenced the dimensions of the emergent landmass with the result that current islands would have been connected, and land area was much more extensive than it is today (Campbell, 1993) (figure 6.2). As a consequence of rising sea levels the animal and plant lineages on the remaining emergent parts of the Chathams became separated by water up to 100m in depth. This is likely to have resulted in lineage sorting and bottleneck effects in the residual populations. The Chatham Islands harbour unique biota with many endemic taxa, but the majority of endemicity is at or below species level (e.g. beetle fauna, Emberson, 1995 & 1998).

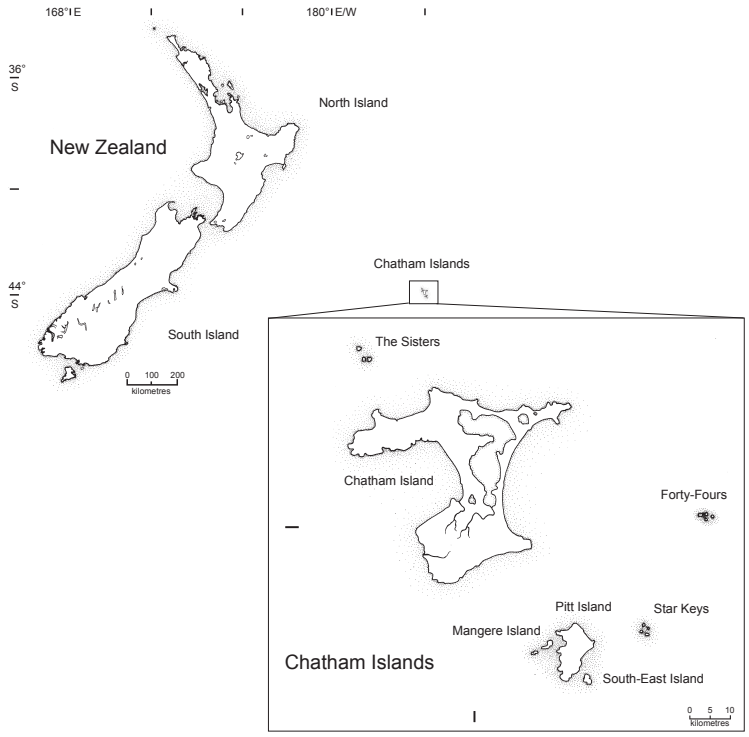


Figure 6.1: Map of New Zealand and the Chatham Islands.

In order to explore whether congruence is evident among taxon groups in their response to past geophysical events, data for lineages respresented in mainland New Zealand and the Chatham Islands were gathered. The Chathams provide a context for studying New Zealand biogeography that is relevant in spatial (two land areas separated by ~800 km of ocean) and temporal (emergence ~ 4 Ma) terms. The geographic distance from mainland New Zealand to the Chathams is about half the length of the main islands of New Zealand (~1600 km), and 4 million years (Myr) is an appropriate time period for exploring species evolution (Hewitt, 2001). Furthermore, this Plio/Pleistocene period was one of significant geophysical activity in New Zealand. Thus, there is a nice contrast of highly disjunct versus near continuous habitat availability. Data from invertebrate taxa in several orders present on the Chatham Islands were examined, with the most intensive sampling placed on a genus of cave weta (Orthoptera: Raphidophoridae) that is widespread on mainland New Zealand and Chatham Islands. These data allow assessment of the degree of congruence among phylogeographic patterns in mitochondrial lineages within this diverse set of invertebrate taxa within and between the Chatham Islands and New Zealand.

VI.2 MATERIAL & METHODS

The invertebrate specimens for this study were collected mainly by hand and preserved in 95% Ethanol. They are housed and catalogued in the collection of the Phoenix Lab at Massey University, Palmerston North with unique voucher numbers. Prior to extraction one leg was removed from each specimen. DNA was extracted using a standard salting-out method (Sunnucks & Hale, 1996). The extractions are stored at -20°C.

A polymerase chain reaction (PCR) was carried out primarily targeting the mitochondrial cytochrome oxodase I and II (COI and COII) gene regions (794 - 890 bp and 595 bp). For amplification the published oligonucleotide primers C1-J-1718 and L2-N-3014 (COI) and TL2-J-3037 and C2-N-3661 (COII) were used (Simon *et al.*, 1994). PCR amplifications were performed in a total volume of 10µl using Red Hot Taq (ABgene). After an initial denaturation at 94°C for 3 min. DNA was amplified during 35 cycles of 30 sec at 94°C, 45 sec at 50°C and 30 sec at 72°C, followed by a final extention step at 72°C for 4 min. For *Talitropsis* sp. the mitochondrial 16S gene region (762 bp) was also amplified using published primers N1-j-12585 and LR-N-13398 (Simon *et al.*, 1994). The PCR cycle conditions varied from above as follows: 35 cycles of 1 min. at 94°C, 1 min. at 42°C and 1.5 min. at 72°C, followed by a final extension step at 72°C for 5 min.

The amplified products were checked on 1% Agarose gels and purified using SAP/EXO1 (USB Corporation) enzyme digest following the manufacturer's instructions. Purified DNA fragments were used for cycle sequencing with Big Dye terminators under standard conditions and read on an ABI 377 sequencer (ABI).

Phylogenetic Analysis

Sequences were edited using Sequencher 4.9 (Gene Codes Corporation) and aligned using SeAl (Rambaut ,1996). Neighbor-Joining (NJ) as well as Maximum Likelihood (ML) analyses were conducted in PAUP* (Swofford, 1998) with the individual datasets to verify the tree topology. It was then used to conduct a ML heuristic search with a NJ starting tree, tree- bisection-reconnection (TBR) and a maximum of 100 trees. Bootstrap values were obtained for NJ with 1000 replicates. Additionally Mr.Bayes 3.1.2 (Ronquist & Huelsenbeck, 2003) was used to examine tree topology with both datasets under a six parameter model selected by jModelTest 3.5 (Guindon & Gascuel, 2003; Posada, 2008). MrBayes implemented a GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites. Four independent MCMC runs for ten million generations with a burn in of 10% were employed. Resulting posterior probabilities on the nodes were recorded.

Population analyses

Uncorrected *p*-distances for all taxa were calculated using PAUP*4 (Swofford, 1998). The program tcs 1.21 (Clement *et al.*, 2000) was run with a 95% connection limit to construct

parsimony haplotype networks of all taxa to assess their geographic structuring the Chatham Islands. For *Talitropsis* a network of the whole genus was constructed including specimens of the mainland New Zealand species *T. sedilloti*. DnaSP v5.0 (Rozas *et al.*, 2003) was used to calculate nucleotide diversity (π, Nei, 1987) and additionally haplotype diversity (*h*) and average number of nucleotide differences (*k*) for the mainland New Zealand cave weta species *T. sedilloti*. Furthermore mismatch distributions for *T. sedilloti* were calculated and a Mantel-test for isolation by distance (IBD) was run using IBDWSv.2 (Jensen *et al.*, 2005).

Taxa

Talitropsis (Orthoptera: Raphidophoridae)

The family Raphidophoridae (called cave weta in New Zealand) comprises approx. 300 species worldwide (Groll & Günther, 2003) with 18 genera endemic to New Zealand. Six of these genera are monotypic and confined to offshore islands. *Talitropsis* on the other hand is an endemic genus consisting of three recognized species. Two allopatric endemic species are found on the Chatham Islands, *T. crassicruris* (Hutton, 1897) and *T. megatibia* Trewick, 1999 and one species is widespread throughout mainland New Zealand, *T. sedilloti* Bolivar, 1882.

Geodorcus (Coleoptera: Lucanidae)

The members of the genus *Geodorcus* (stag beetles) are relatively rare and flightless and have a comparatively large body size. There are 24 described stag beetle species in New Zealand with *Geodorcus* comprising ten species (Holloway, 1961). Two endemic species are found on the Chatham Islands *Geodorcus capito* Deyrolle, 1873 and *G. sororum* Holloway, 2007.

Hadramphus (Coleoptera: Curculionidae)

The weevil genus *Hadramphus* comprises five species with four being endemic to New Zealand and one being endemic to the Chatham Islands (*H. spinipennis* (Broun, 1911)). Taxonomically they belong to the tribe Molytini which includes another 16 species of the genus *Lyperobius* in New Zealand (Craw, 1999). The *Hadramphus* species are relatively large flightless weevils (11.7-23 mm). Both adults and larvae feed on the plants of the families Apiaceae, Araliaceae and one on Pittosporaceae and are therefore restricted to the distribution of these plants. On the Chatham Islands *H. spinipennis* feeds and lives on the endemic speargrass *Aciphylla dieffenbachii.* All of the species are endangered these days and are mainly confined to offshore islands or remote areas in Fiordland, South Island. One species (*Hadramphus tuberculatus* (Pascoe, 1877)) was last collected in 1922 in the Canterbury region and was thought to be extinct. Fortunately it was rediscovered in 2004 and legs of two specimens were made available for this study. One other species (*H. stilbocarpae* (Kuschel, 1971)) that was included in the study can be found in Fiordland and Southland of New Zealand.

These beetles were chosen because they have no obvious dispersal traits and are limited to particular food sources. However, two problems arise when dealing with endangered, rare

taxa. First of all it is almost impossible to collect a large sample number to get an insight into the population genetics and variability within the species/genus. Secondly it is often impossible to find true closest relatives of populations of interest as there is a high likelihood that target species have recently gone extinct.

Amychus (Coleoptera: Elateridae)
Amychus are very large, flightless click beetles. As with *Hadramphus* all its species are endangered and are restricted to small predator-free offshore islands. The genus is endemic to New Zealand. Three extant species are known from New Zealand and one is endemic to the Chatham Islands. The two species from mainland New Zealand, one from Three Kings Island *Amychus manawatawhi* (Marris & Johnson, 2010) and *Amychus granulates* Broun, 1883 is now restricted to islands in Cook Strait, South Island, were incorporated into the study. The endemic species present on the Chatham Islands *Amychus candezei* Pascoe, 1876 was also included from all significant islands except Pitt and Mangere Islands.

Anisolabis (Dermaptera: Labiduridae)
Anisolabis Fieber 1853 is an earwig genus distributed around the Pacific. *Anisolabis littorea* (White, 1846) is an endemic species that can be found under rocks and logs on beaches around New Zealand and islands, including the Chathams (Hudson, 1973). There are two other species known from New Zealand. *A. kaspar* (Hudson, 1972) is endemic to the Three Kings Islands and *A. occidentalis* has been introduced from Australia, but is currently restricted to the Hawkes Bay coast, eastern North Island. A sibling species *A. maritima* that occurs around the Pacific but not New Zealand was included in this study.

Tables with sampling location and voucher numbers for all taxa employed in this study can be found in the Appendix (Table S6.1).

VI.3 RESULTS

Cave weta
The sampling of *Talitropsis* cave weta included all three recognized species of the genus. The two species on the Chathams are currently isolated from one another with *T. crassicruris* inhabiting Main Island and The Sisters and *T. megatibia* the southern islands of the archipelago including the 44s. The widespread *T. sedilotti* on the other hand can be found everywhere in mainland New Zealand, including both main islands and several small offshore islands (figure 6.2). A set of 96 specimens comprising all three species of *Talitropsis* plus three samples of the putative closest sister taxon within New Zealand (*Isoplectron*) as outgroup were sequenced for a 875bp long fragment of COI. The phylogenetic reconstruction of the genus showed that the species form monophyletic clades (figure 6.2a). The same overall grouping is apparent

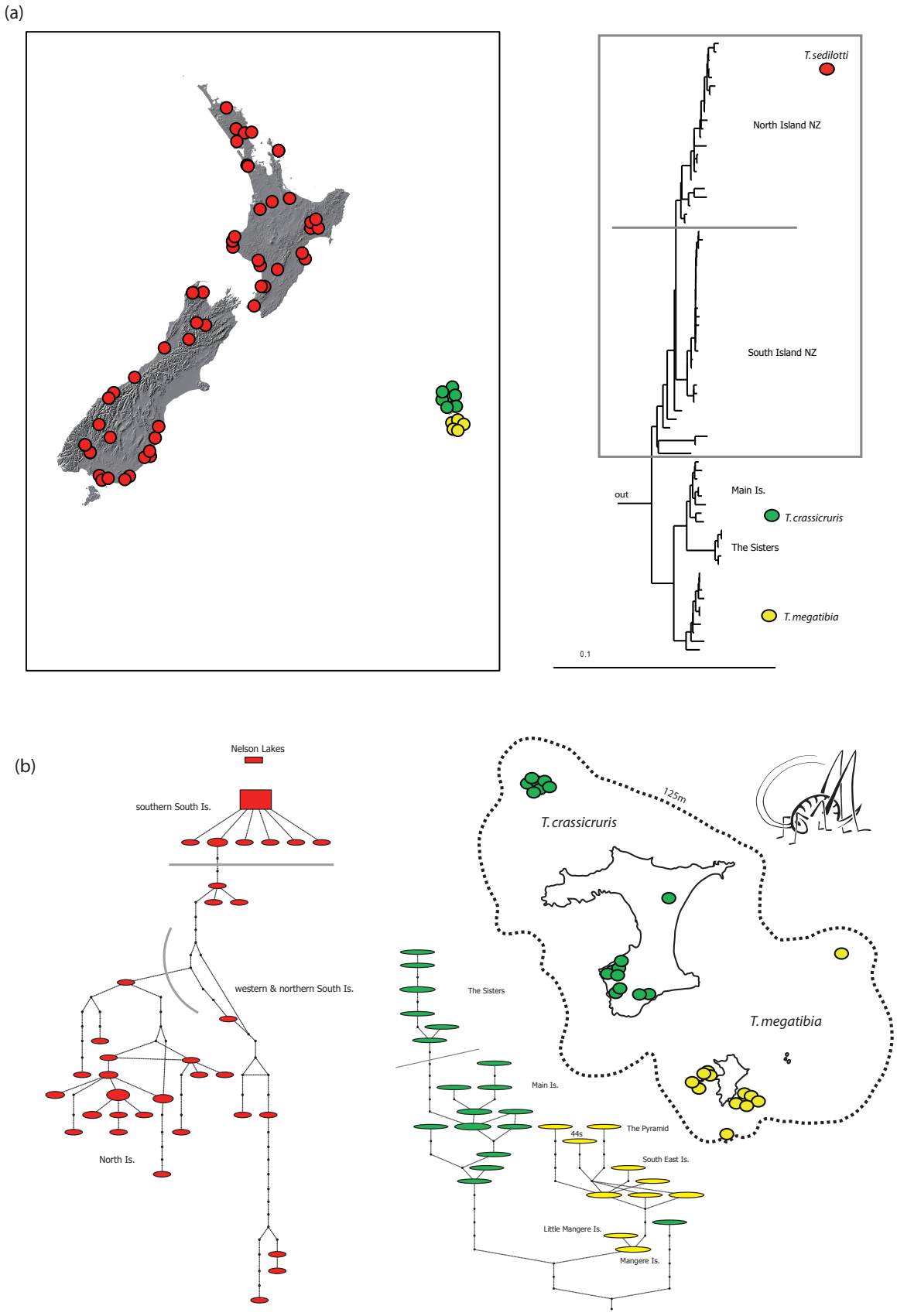


Figure 6.2: Map of New Zealand and the Chatham Islands with mapped sampling location of the three species of *Talitropsis*, including phylogenetic reconstructions of the genus (a) and haplotype networks (b) based on the COI dataset. Coloured circles in (b) show haplotypes encountered and small black circles on branches stand for missing haplotypes; colours in both figures correspond to the three different species of *Talitropsis*.

in the 16S dataset (not shown). A single haplotype network was generated for *T. sedilotti* and one for the two Chatham species (figure 6.2b) with the program TCS (95% connection limit). *T. sedilotti* samples exhibit some regional structuring with samples from North Island and South Islands grouping together, although no fine scale structuring is evident. Within the Chatham species the network is split into the two species. *T. crassicruris* shows division between population of the Sisters and Main Island, but there is no such structure apparent within individuals of *T. megatibia* in the southern part of the archipelago (yellow circles). Mismatch distributions calculated for *T.sedilotti* highlight the frequency of genetic divergence within this species (figure 6.3a). Furthermore the Mantel-test for IBD showed that there was no correlation between genetic and geographic distances in this species (figure 6.3b). Population genetic statistics calculated for the different species of *Talitropsis* showed that the nucleotide diversity (π) is similar between the New Zealand species *T. sedilotti* and the Chatham population and that within the Chatham species π is much lower (Table 6.1). The haplotype diversity (*h*) within and between species on the other hand is more or less similar. Genetic pairwise distances within *T. sedilotti* was up to 3.12% and almost as high as the distance between the two endemic Chatham species with 3.4%. The genetic distance within these two species was up to 2.38% in *T. crassicruris* and 0.98% in *T. megatibia*. Between the Chathams and New Zealand taxa the genetic distances ranged from 1.59 to 4.67%.

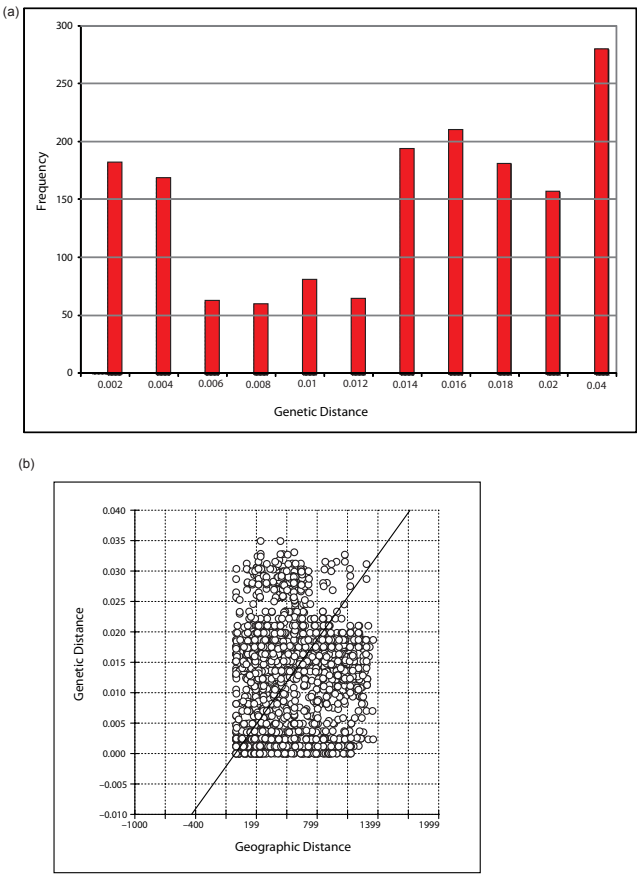


Figure 6.3: (a) Mismatch distribution plot calculated with frequencies and pairwise genetic distances of *T. sedilotti*. (b) Result of a Mantel-test for isolation by distance (IBD)for *T. sedilotti* samples using IBDWSv.2 (Jensen *et al.*, 2005).

Table 6.1: DNA variation and haplotype diversity within and between species of *Talitropsis* in the New Zealand region, with the sample size for each region (*n*), number of observed haplotypes (N_{haps}), average number of nucleotide differences (*k*), nucleotide diversity (π), and haplotype diversity (*h*).

Area	*n*	N_{haps}	*k*	π	*h*
T. sedilotti	56	33	8.48636	0.01284	0.948
T. crassicruris	22	17	5.38095	0.00814	0.974
T. megatibia	13	10	4.29487	0.00650	0.962
Ch.Is. population	35	27	8.35630	0.01264	0.985

Stag beetles

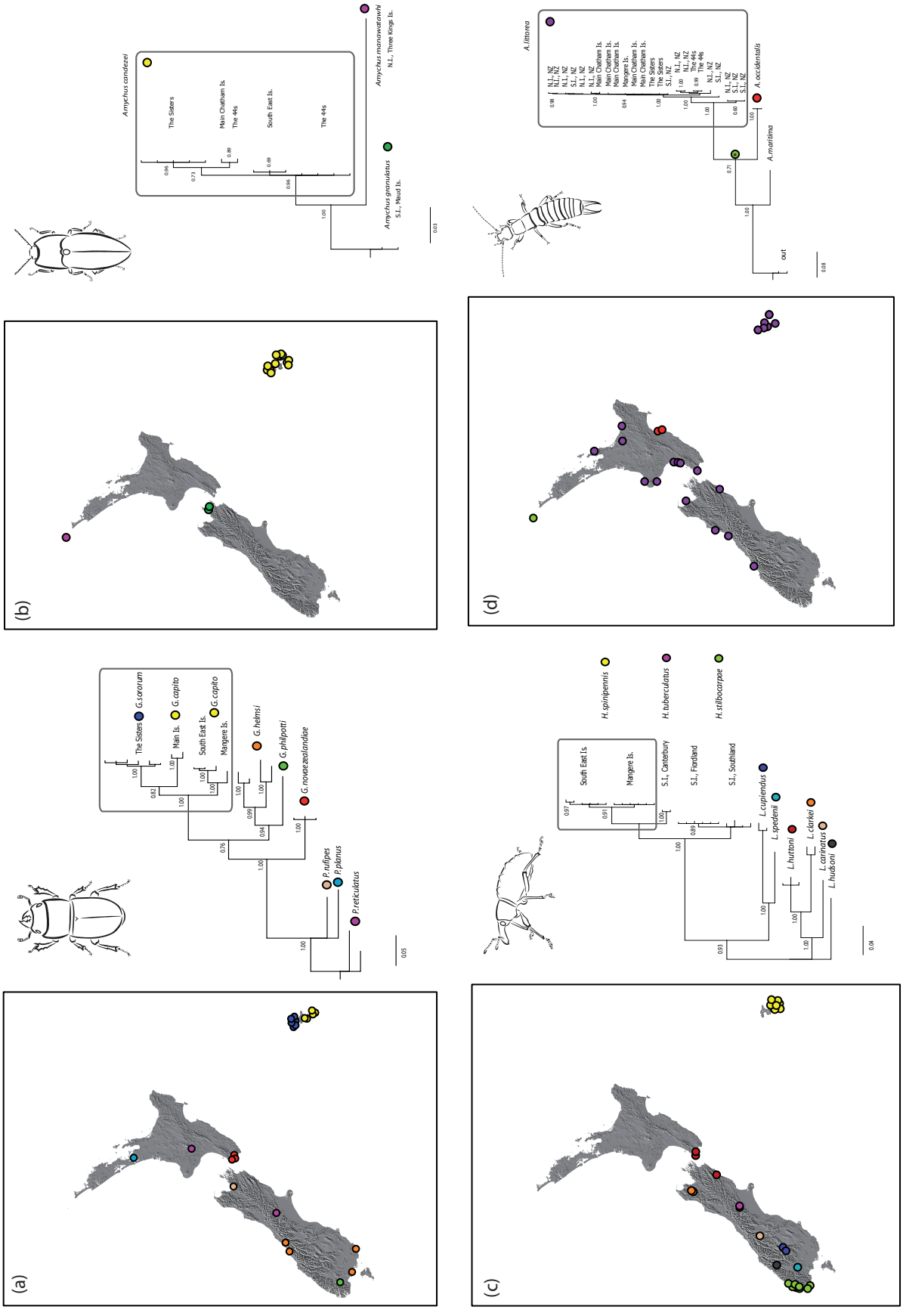
For *Geodorcus* stag beetles a 777bp long fragment of the mitochondrial COI region was sequenced and used to construct a Bayesian phylogenetic tree and a haplotype network. The Bayesian tree included the two endemic species from the Chatham Islands and three species found in New Zealand. Additionally three species of the closest sister of *Geodorcus* in New Zealand, *Paralissotes*, were included as outgroup (figure 6.4a). Specimens of *Geodorcus sororum*, that is reported only from The Sisters, form a monophyletic clade, but specimens of the other species *G. capito* sampled from Main Is., South East Is. and Mangere Is., formed two clades. The Main Is. population appears to have closer affinities to the species from The Sisters. This is highlighted in the haplotype network (figure 6.5a), with *G. capito* falling into separate clusters corresponding with populations on different islands at a 95% connection limit (in fact, even with a 90% connection limit it was not possible to connect the Main Is. population to the other populations of *G. capito*). The genetic distances among *G. sororum* haplotypes were 0.23-0.96%. Within the different populations of *G. capito* genetic distances were between 0.0 and 6.9% and between the two endemic species 4.28-6.75%.

Clickbeetle

A 784bp fragment of COI was sequenced for three species of the genus *Amychus*. The endemic species *Amychus candezei* was collected on four islands within the Chatham archipelago. Additionally one specimen of the endemic species from the Three Kings Is. (*A. manawatawhi)* and two of *A. granulates* from northern South Island were included. Bayesian posterior probabilities were mapped on branches (figure 6.4b) and the haplotype network of *A. candezei* was constructed with a 95% connection limit and colours correspond to species (figure 6.5b). The genetic diversity within the Chatham species is was up to 1.2% and between species up to 9.6 % (*A. manawatawhi*) and up to 4.7% (*A. granulates*).

Weevil

The genus *Hadramphus* comprises four species in New Zealand and three were available for this study. One species (*H. spinipennis*) is endemic to the Chatham Islands and can be found on Mangere and South East Island, were its host plant (*Aciphylla dieffenbachii*) is established. An 816bp fragment of COI was sequenced for the three species of *Hadramphus* plus additional six species of its closest sister taxon in New Zealand (*Lyperobius*) were included. Not surprisingly, the Bayesian tree revealed distinct monophyletic groups according to species (figure 6.4c) as did the haplotype network (not including *H. tuberculatus,* figure 6.5c). Genetic distances within the Chatham species were 0-0.5%, compared to the southern South Island species with 0-0.9%. Genetic distances between New Zealand and Chatham species was between 2.7 and 4.0%.



[Fig. 6.4: Figure legends can be found on the following page]

Figure 6.4: Map of New Zealand with sampling location and Bayesian tree reconstruction, for taxa employed in this study; Bayesian posterior probabilities are mapped mapped on branches
(a) *Geodorcus*; the phylogenetic reconstruction included *Geodorcus* and *Paralissotes* specimens from mainland New Zealand, the coloured circles on the tree correspond to circles on the map of New Zealand, depicting sampling locations; the grey box highlights the two endemic Chatham Island species.
(b) *Amychus*; the Bayesian phylogenetic reconstruction included the endemic Chatham Is. species and two species from mainland New Zealand; coloured circles corresponding to species and sampling locations.
(c) Molytini; the phylogenetic reconstruction included *Hadramphus* weevils from mainland New Zealand and the Chathams and *Lyperobius* specimens from mainland New Zealand; coloured circles correspond to species and sampling locations; the grey box highlights the endemic Chatham Island species.
(d) *Anisolabis*, the phylogenetic reconstruction included *Anisolabis* earwigs, including the widespread New Zealand endemic *A. littorea* (grey box), that can be found in coastal habitat around New Zealand and the Chatham Islands, the localize introduced species *A. occidentalis* and the cosmopolitan species A. maritime. The green circle indicates the putative position of the second New Zealand endemic species *A. kaspar*, that is only found on the Three Kings Is. in northern New Zealand (green circle in map); coloured circles correspond to species and sampling locations.

Earwig

Specimens of the widespread earwig species *Anisolabis littorea* and several sister taxa were sequenced for a 1500bp fragment of COI and COII (figure 6.4d). For *A. kaspar* unique primer pairs covering COI fragments were designed for short overlapping fragments (Table S6.2) and phylogenetic reconstruction including this species just with COI placed it as sister to *A. littorea* (green circle). Not surprisingly the Chatham Islands specimens formed one haplotype network with the New Zealand samples, but expressed several unique haplotypes (figure 6.5d). Genetic distances within the Chatham Island samples was up to 1.2% and between mainland New Zealand specimens and Chathams up to 2.9%.

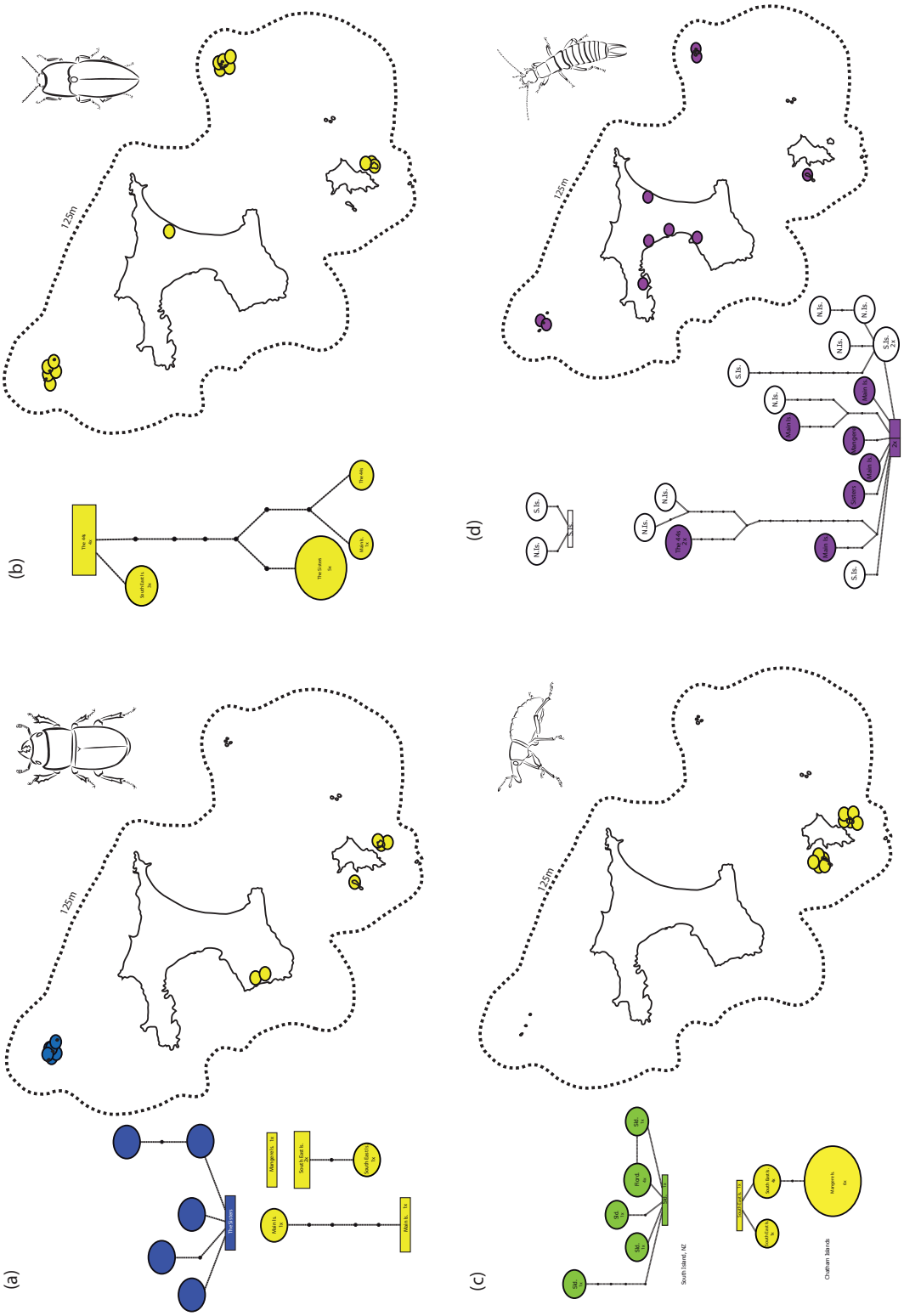


Figure 6.5: Map of the Chatham Islands, with 125m bathymetry outline, corresponding to estimated continuous land surface above sea level during the last glacial maximum, showing sampling locations in the Chathams and COI haplotype network for each taxon.
(a) *Geodorcus*; The haplotype network shows the relationship within the Chatham Islands species, with colours corresponding to the two endemic species (*G. sororum*, blue and *G. capito*, yellow).
(b) *Amychus*; The haplotype network shows the relationship within the Chatham Islands (yellow circles).
(c) Molytini; The haplotype networks show the relationship within the Chatham Island endemic species *H. spinipennis* (yellow circles) and the mainland New Zealand haplotypes of *H. stilbocarpae* (green circles); Fiord= Fiordland, Sld=Southland.
(d)) *Anisolabis,* the haplotype network depicts the relationship between the specimens of *A. littorea* from mainland New Zealand and the Chatham Is. (coloured circles).

VI.4 DISCUSSION

One prediction widely made by biogeographers is that distribution patterns and phylogeographic structure of taxon groups are likely to be congruent where they are the product of the same geophysical history. For example, the emergence of the Chatham Island archipelago from the southern ocean about 4 Ma might be expected to result in a set of Chatham Island endemic taxa that were equally divergent from neighbouring New Zealand populations from which they were derived. Studies published to date estimate genetic divergence between terrestrial New Zealand and Chatham Island populations ranging from identical fern cpDNA haplotypes (*Asplenium hookerianum,* Shepherd *et al.*, 2009) to plants with 6.4% sequence divergence (Heenan *et al.*, 2010) and up to 2% sequence divergence in *Oligosoma* skink (Liggins *et al*., 2008). They also include four flightless insect genera (Trewick, 2000), *Anoteropsis* wolf spiders (Vink & Paterson, 2003), Kikihia cicada (Arensburger *et al.*, 2004), Celatoblatta cockroach (Chinn & Gemmell, 2004), *Paracorophium* amphipod (Stevens & Hogg, 2004), *Austridotea* isopods (McGaughran *et al.*, 2006), 16 plant and nine animal genera (Paterson *et al.*, 2006), *Austrolestes* damselfly (Nolan *et al.*, 2007) stick insect *Argosarchus horridus* (Trewick *et al.,* 2005), robin (Miller & Lambert, 2006), rails (Trewick, 1997), pigeons (Goldberg *et al*., 2011) and parakeets (Boon *et al.*, 2001 & 2008). Molecular dating of divergence times, where calculated, has been in the range of 0–7 Myr. Given the wide taxonomic and ecological diversity represented here, this range of divergence estimates might be attributed to both history and biology. However, even when flightless forest insects are compared one finds little evidence of congruence; cave weta and click beetles provide estimates of between 4.6 and 9.6% of genetic diversity between New Zealand and the Chatham Islands. Despite the use of rapidly evolving DNA sequence marker (mitochondrial gene regions), all examined invertebrate taxa consistently show genetic distances between species from mainland New Zealand and their closest relatives on the Chatham Islands, that are too low to be explained by ancient separation. These results confirm earlier studies concerning the origin and age of several Chatham invertebrate taxa (Trewick, 2000). These low genetic distances are consistent with relatively recent colonization of the islands and splitting of lineages in the Pliocene (5–2 Myr) as predicted by current geology (Campbell, 1998; Campbell *et al.*, 2006). Studies that have explored phylogeographic structure of single species that span the New Zealand and Chathams range reveal, not surprisingly, low genetic distances, but also evidence for the transport of multiple genotypes (e.g. *Asplenium hookerianum* - Shepherd *et al.*, 2009; *Phaulacridium* - chapter V; *Anisolabis* - this study). This might reflect colonization by a large group of individuals at one time or sustained gene flow over time.

The mixture of phylogeographic patterns observed within the Chathams is similar to that seen on the larger spatial scale. No single pattern was detected when flightless insects are compared (figures 6.5a-d). While population structuring within the Chathams might not be expected for taxa that are not structured between New Zealand and Chathams (e.g. *Phaulacridium* - chapter V**,** *Anisolabis*), those that are partitioned at the source could show congruent spatial structure at

a fine scale. This does not appear to be so, however, the fact that some of these and other animals have endemic taxa in the Chathams is itself striking (e.g. pigeon - chapter IV, *Talitropsis*). Indeed, some lineages have more than one species and where this is the case they are allopatric (e.g. *Talitropsis*) and sometimes paraphyletic (e.g. *Geodorcus*).

Given the young geological age for the islands and much younger age for land connection (through Pleistocene) the instance of multiple endemics is intriguing, especially where this contrasts with the situation of sibling taxa in New Zealand. For instance *Talitropsis* genetic diversity in New Zealand and Chathams are about the same, yet the land areas and ages are vastly different. Therefore, other drivers of diversity are implicated. This is born out by the respective distribution of diversity, which in the case of *Talitropsis* is indicative of recent southwards range expansion in New Zealand (figure 6.2), whereas in the Chathams, genetic diversity is tightly partitioned over a narrow spatial scale across 50 km of small islands. This is despite the fact that New Zealand and Chathams would each have consisted of one main land area during the LGM and at earlier periods of lowered sea level.

The observation that land area and age might not be accurate predictors of genetic or taxonomic diversification is significant because these are the traits that are routinely used in biogeographic analyses. Similarly, the demonstration that not only are taxa with "good" dispersal characteristics (e.g. pigeon – Goldberg *et al*., 2011, rail – Trewick, 1997; fern spores - Shepherd *et al.*, 2009) able to reach islands such as the Chathams, but so are taxa that apparently lack dispersal attributes. In the present study, the presence of flightless insects in the Chathams that are sister to flightless species in New Zealand demonstrates that such animals are not prevented from oversea dispersal. This suggests that involuntary mechanisms operate. It might be relevant for instance, that all the taxa studied spend a substantial amount of their lives in logs, and logs are known to be transported down rivers and to the sea, and to arrive on beaches having drifted in the ocean sometimes carrying animals and plants with them (Johansen & Hytteborn, 2001). New Zealand *Anisolabis* earwigs are most readily found in and around drift wood on beaches, stage beetle larvae and pupa live and feed in logs, and *Talitropsis* cave weta of all ages occupy holes in wood. Interestingly, in the Chathams the opportunities for log dwelling are fewer and on some islands especially (44s, The Sisters) trees are absent. Here, *Talitropsis* and *Geodorcus* occupy cavities in the peaty soil formed between rocks. Perhaps Carlquist's (1974) inference that dispersal traits may be lost on islands for many plants extends to insects.

Finding biogeographic congruence among taxa, at small or large geographic distances, with or without obvious habitat distinctions and in a time frame that encompasses substantial geogphysical changes, has been proven difficult. Two important conclusions arise from this. The first is that if hypotheses about the influence on the partitioning of biodiviersity based on land size, age, distribution etc. cannot be upheld when data are available, inferences made about the biogeography of taxa without data must be treated very tentatively. Secondly, if such factors are not the most important predictors of phylogeographic and phylogenetic partitioning, then a

considerable amount of work is needed to describe ecological and behavioural traits of plants and animals so that tests for their biogeographic influence can be made. Furthermore, these data are required to refute the hypothesis that stochastism is the primary force in biogeography.

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APPENDIX CHAPTER VI

Table S6.1: Tables of taxa and sampling location in this study; (a) *Talitropsis*

Sample ID	Species	Location	Coordinates
CW48.1	*T. sedilloti*	N.I.,Hawkes Bay,Mohi Bush	39 39 52 56 S / 176 58 21 33 E
CW47.1	*T. sedilloti*	N.I.,Hawkes Bay,Hastings,Mohi Bush	39 39 52 56 S / 176 58 21 33 E
Tsed4	*T. sedilloti*	S.I., Dunedin, Frasers Bush	45 51 42 33 S / 170 27 29 77 E
Tsed7	*T. sedilloti*	N.I.,Northland,Whatitiri Scenic Res.	35 47 35 25 S / 174 07 59 31 E
Tsed8	*T. sedilloti*	S.I.,Takaka,The Grove	40 50 55 06 S / 172 52 07 66 E
Tsed9	*T. sedilloti*	S.I.,Nelson,Nelson Lakes, Mt. Roberts, Carpark	41 50 03 29 S / 172 48 48 80 E
Tsed24	*T. sedilloti*	S.I.,Te Anau,Rainbow Reach	45 27 19 05 S / 167 41 39 88 E
Tsed26	*T. sedilloti*	S.I.,Catlins,Matai Falls	46 29 55 25 S / 169 29 31 64 E
CW209.1	*T. sedilloti*	N.I.,Te Urewera,Lake Waikaremoana, Hinerau Walk	38 47 31 64 S / 177 07 36 52 E
CW211	*T. sedilloti*	N.I.,Waikato,Waitomo Caves,Short Bush Walk	38 16 11 97 S / 175 05 16 76 E
CW210	*T. sedilloti*	N.I.,BOP,nr. Mangatoi,Otanewainuku Forest,Rimu Tr.	37 54 18 43 S / 176 12 17 27 E
CW207	*T. sedilloti*	N.I.,Mt Taranaki,East-Taranaki,Patea Track	39 19 32 73 S / 174 06 26 97 E
CW482.1	*T. sedilloti*	S.I., Southland, Takitimu Ra., Pinchester Bush	45 40 57 99S / 167 53 01 49E
CW482.2	*T. sedilloti*	S.I., Southland, Takitimu Ra., Pinchester Bush	45 40 57 99S / 167 53 01 49E
CW482.3	*T. sedilloti*	S.I., Southland, Takitimu Ra., Pinchester Bush	45 40 57 99S / 167 53 01 49E
CW21	*T. sedilloti*	N.I., Auckland, Waitakere, Opanuku Rd	36 54 37 83 S / 174 34 04 72 E
CW23	*T. sedilloti*	N.I., Northland,Prescot Rd nr Ruakaka	35 54 56 69 S / 174 27 14 97 E
CW29	*T. sedilloti*	N.I.,Auckland,Waitakere,Arataki	36 56 56 52 S / 174 36 26 17 E
CW275	*T. sedilloti*	S.I., Otago, Leith Valley, Dunedin	45 50 07 14 S / 170 29 41 53 E
CW20	*T. sedilloti*	N.I.,Northland, Hukatere	36 10 41 92 S / 174 10 04 87 E
CW331	*T. sedilloti*	N.I., Coromandel, Cuvier Island	36 26 20 67 S / 175 46 14 02 E
CW350	*T. sedilloti*	S.I., Nelson Lakes NP, Lake Rotoroa, loop Track	41 47 39 14 S / 172 35 58 23 E
CW351	*T. sedilloti*	S.I., Lewis Pass NP, Lake Daniells Track	42 18 11 88 E / 172 17 05 78 E
CW352	*T. sedilloti*	S.I., Fiordland NP, Te Anau, Kepler Track	45 26 22 00 S / 167 41 05 02 E
CW353	*T. sedilloti*	S.I., Fiordland NP, Te Anau, Kepler Track	45 26 18 53 S / 167 40 47 58 E
CW354	*T. sedilloti*	S.I., Otago, Queenstown, Kinloch	44 50 34 19 S / 168 20 58 55 E
CW355	*T. sedilloti*	S.I., Otago, Catlins Coast, Papatowai	46 34 08 60 S / 169 28 16 12 E
CW45.1	*T. sedilloti*	N.I.,Te Urewera, L. Waikaremoana, Black Beech Track	38 46 11 19 S / 177 08 15 34 E
CW276	*T. sedilloti*	S.I., Otago, Hampden, Kurinui	45 19 48 47 S / 170 45 46 30 E
CW371	*T. sedilloti*	N.I., Wellington, Eastbourne	41 17 49 69 S / 174 54 00 85 E
CW377	*T. sedilloti*	N.I., Waikato, Te Awamutu, Maungatautari	38 01 08 81 S / 175 34 33 59 E
CW423	*T. sedilloti*	N.I., Wanganui	39 57 40 07 S / 175 03 05 40 E
CW457	*T. sedilloti*	N.I., Lady Alice Island	35 53 25 77 S / 174 43 46 27 E
Tsed21	*T. sedilloti*	S.I.,Invercargill, Otarara Scenic Res.	46 31 44 11 S / 168 15 26 59 E
Tsed22	*T. sedilloti*	S.I.,Central Otago, nr. Beaumont	44 58 51 52 S / 170 55 33 95 E
Tsed23	*T. sedilloti*	S.I.,Westland, Haast River	43 52 59 59 S / 169 02 55 21 E
CW128	*T. sedilloti*	N.I., Northland	35 08 40 90 S / 173 45 37 78 E
CW428	*T. sedilloti*	N.I., Wanganui	39 57 42 76 S / 175 02 43 43 E
CW469	*T. sedilloti*	N.I., Taranaki	39 16 05 23 S / 174 03 01 85 E
CW499.2	*T. sedilloti*	S.I., Invercargill, Bluff Scenic Res.	46 36 51 98 S / 168 20 42 06 E
CW499.1	*T. sedilloti*	S.I., Invercargill, Bluff Scenic Res.	46 36 51 98 S / 168 20 42 06 E
CW356.1	*T. sedilloti*	S.I.,Kahurangi NP, Golden Bay,Start of Heaphy Track	40 51 38 73 S / 172 27 06 00 E
CW356.3	*T. sedilloti*	S.I.,Kahurangi NP,Golden Bay,Start of Heaphy Track	40 51 38 73 S / 172 27 06 00 E
CW356.2	*T. sedilloti*	S.I.,Kahurangi NP,Golden Bay,Start of Heaphy Track	40 51 38 73 S / 172 27 06 00 E
CW209.2	*T. sedilloti*	N.I.,Te Urewera,Lake Waikaremoana, Hinerau Walk	38 47 31 64 S / 177 07 36 52 E
CW209.3	*T. sedilloti*	N.I.,Te Urewera,Lake Waikaremoana, Hinerau Walk	38 47 31 64 S / 177 07 36 52 E

CW274	*T. sedilloti*	S.I., Otago, Leith Valley, Dunedin	45 50 23 40 S / 170 30 10 23 E
CW469.2	*T. sedilloti*	N.I., Taranaki	39 16 05 23 S / 174 03 01 85 E
CW358.1	*T. sedilloti*	N.I., Levin, Tararua Ra., Gladstone Rd, Makahika Lodge	40 38 48 78 S / 175 22 42 59 E
CW160	*T. sedilloti*	N.I., Manawatu, Levin, Papaitonga Reserve	40 38 36 09 S / 175 14 00 16 E
CW5	*T. sedilloti*	S.I.,West Coast, Lake Matheson	43 26 22 05 S / 169 57 45 82 E
CW35	*T. sedilloti*	S.I.,South of Haast Beach,Okuru River	43 55 04 80 S / 168 53 36 36 E
CW191	*T. sedilloti*	S.I., Lake Wakatipu, Te Kere Haka Reserve	45 19 29 60 S / 168 42 50 26 E
CW124	*T. sedilloti*	S.I., Westland, Kumara Junction	42 35 07 12 S / 171 07 46 82 E
CW469.1	*T. sedilloti*	N.I., Taranaki	39 16 05 23 S / 174 03 01 85 E
CW208	*T. sedilloti*	N.I.,Manawatu, Pohangina Valley, Camp Rangi Woods	40 07 47 25 S / 175 51 03 49 E
CW481	*T. sedilloti*	N.I., Coromandel, Cuvier Island	36 26 20 67 S / 175 46 14 02 E
CW481z	*T. sedilloti*	N.I., Coromandel, Cuvier Island	36 26 20 67 S / 175 46 14 02 E
CW83	*T.crassicruris*	Ch.Is., Main Is., Tuku Reserve, Taiko Camp	44 04 20 47 S / 176 38 10 36 W
CW102.1a	*T.crassicruris*	Ch. Is., Main Is., Awatotora	43 59 35 37 S / 176 37 15 63 W
CW102	*T.crassicruris*	Ch. Is., Main Is., Awatotora	43 59 35 37 S / 176 37 15 63 W
CW102.2	*T.crassicruris*	Ch. Is., Main Is., Awatotora	43 59 35 37 S / 176 37 15 63 W
CW102.3	*T.crassicruris*	Ch. Is., Main Is., Awatotora	43 59 35 37 S / 176 37 15 63 W
CW101	*T.crassicruris*	Ch. Is., Main Is., Awatotora	43 59 35 41 S / 176 37 15 64 W
CW101.1	*T.crassicruris*	Ch. Is., Main Is., Awatotora	43 59 35 41 S / 176 37 15 64 W
CW101.2‘	*T.crassicruris*	Ch. Is., Main Is., Awatotora	43 59 35 41 S / 176 37 15 64 W
CW104	*T.crassicruris*	Ch. Is., Te Whanga Lagoon, Te Mataroe Bush	43 54 59 29 S / 176 28 43 49 W
CW216	*T.crassicruris*	Ch. Is.,The Sisters,Middle Sister	43 34 14 34 S / 176 48 39 60 W
CW216.1	*T.crassicruris*	Ch. Is.,The Sisters,Middle Sister	43 34 14 34 S / 176 48 39 60 W
CW216.2	*T.crassicruris*	Ch. Is.,The Sisters,Middle Sister	43 34 14 34 S / 176 48 39 60 W
CW212	*T.crassicruris*	Ch. Is.,Main Is.,Hapupu Reserve	43 48 08 68 S /176 20 59 74 W
CW214	*T.crassicruris*	Ch. Is.,Main Is.,Southern Tablelands	44 05 48 01 S / 176 32 07 48W
CW214.1	*T.crassicruris*	Ch. Is.,Main Is.,Southern Tablelands	44 05 48 01 S / 176 32 07 48W
CW205	*T. megatibia*	Ch. Is.,South East Is.	44 21 03 92 S / 176 10 10 47 W
CW204	*T. megatibia*	Ch. Is.,South East Is.	44 21 03 92 S / 176 10 10 47 W
CW357	*T. megatibia*	Ch. Is., The Pyramid, Camp Flat	44 25 56 51S / 176 14 24 98 W
CW203.1	*T. megatibia*	Ch. Is.,Mangere Is.,Robin Bush	44 16 27 56 S / 176 18 03 46 W
CW215	*T.crassicruris*	Ch. Is.,The Sisters,Middle Sister	43 34 18 29 S / 176 48 31 77 W
CW218	*T.crassicruris*	Ch. Is.,Main Is.,Tuku Reserve	44 03 47 02 S / 176 37 35 00 W
CW02	*T.crassicruris*	Ch. Is.,Main Is.,Whakamarino	43 47 04 72 S / 176 32 53 84 W
CW213	*T. megatibia*	Ch. Is.,The 44s	43 57 54 30 S / 175 49 48 88 W
CW219.1	*T. megatibia*	Ch. Is.,South East Is.	44 20 29 19 S / 176 10 24 23 W
CW219.2	*T. megatibia*	Ch. Is.,South East Is.	44 20 29 19 S / 176 10 24 23 W
CW219.3	*T. megatibia*	Ch. Is.,South East Is.	44 20 29 19 S / 176 10 24 23 W
CW206.1	*T. megatibia*	Ch. Is,Mangere Is.,Robin Bush	44 16 27 56 S / 176 18 03 46 W
CW206.2	*T. megatibia*	Ch. Is,Mangere Is.,Robin Bush	44 16 27 56 S / 176 18 03 46 W
CW217.1	*T.crassicruris*	Ch. Is.,The Sisters,Middle Sister	43 34 14 40 S / 176 48 39 60 W
CW217.3	*T.crassicruris*	Ch. Is.,The Sisters,Middle Sister	43 34 14 40 S / 176 48 39 60 W
CW217.2	*T.crassicruris*	Ch. Is.,The Sisters,Middle Sister	43 34 14 40 S / 176 48 39 60 W
CW8	*T. megatibia*	Ch. Is.,South East Is.	44 21 03 92 S / 176 10 10 47 W
CW7	*T.crassicruris*	Ch. Is.,Main Is.,Whakamarino	43 47 04 72 S / 176 32 53 84 W
Cw13	*T. megatibia*	Ch. Is.,Little Mangere Is.	44 17 11 31 S / 176 20 08 44 W
CW14	*T. megatibia*	Ch. Is.,Little Mangere Is.	44 17 11 31 S / 176 20 08 44 W
CW156	Isoplectron sp.		
CW325	Isoplectron sp.		
CW366	Isoplectron sp.		

(b) *Geodorcus & Paralissotes*

Sample ID	Species	Location	Coordinates
SB 03	*G. capito*	Ch. Is., South East Is.	44 20 29 19 S / 176 10 24 23 W
SB 06	*G. capito*	Ch. Is., Taiko Camp,Mt. Albert	44 04 20 47 S / 176 38 10 36 W
SB 25	*G. capito*	Ch. Is., South East Is., Woolshed	44 21 03 80 S / 176 09 59 32 W
SB 41	*G. capito*	Ch. Is., Mangere Is., Robin Bush	44 15 58 43 S / 176 17 20 61 W
SB 42	*G. capito*	Ch. Is., South East Is., Woolshed	44 21 03 92 S / 176 10 10 47 W
SB 47	*G. capito*	Ch. Is.,Main Is.,Tuku Reserve	44 03 47 02 S / 176 37 35 00 W
SB 02	*G. sororum*	Ch. Is., The Sisters	43 34 05 26 S / 176 48 10 61 W
SB 24	*G. sororum*	Ch. Is., The Sisters	43 34 05 26 S / 176 48 10 61 W
SB 46	*G. sororum*	Ch. Is.,The Sisters,Middle Sister	43 34 18 29 S / 176 48 31 77 W
SB 46.1	*G. sororum*	Ch. Is.,The Sisters,Middle Sister	43 34 18 29 S / 176 48 31 77 W
SB 46.2	*G. sororum*	Ch. Is.,The Sisters,Middle Sister	43 34 18 29 S / 176 48 31 77 W
SB 54	*G. sororum*	Ch. Is.,The Sisters,Middle Sister	43 34 17 35 S / 176 48 30 03 W
SB 29.1	*G. philpotti*	S.I., Southland, Borland Saddle	45 49 13 54 S / 167 23 56 54 E
SB 27	*G. novaezealandiae*	N.I., Wellington, Catchpole	41 19 35 21 S / 174 59 06 55 E
SB 72.1	*G. novaezealandiae*	N.I., Wellington, Eastbourne, Butterfly Creek	41 17 45 49 S / 174 54 36 13 E
SB 72.2	*G. novaezealandiae*	N.I., Wellington, Eastbourne, Butterfly Creek	41 17 45 49 S / 174 54 36 13 E
SB 01	*G. helmsi*	S.I., West Coast, Copeland Track	43 32 59 72 S / 169 58 52 41 E
SB 04	*G. helmsi*	S.I., Salmon Farm,North of L. Paringa	43 42 03 56 S / 169 26 17 11 E
SB 21	*G. helmsi*	S.I., Southland, Riverton, Mores Scenic Res.	46 21 44 10 S / 167 59 24 41 E
SB 69	*G. helmsi*	S.I., Otago, Catlins Coast, Papatowai	46 35 01 20 S / 169 25 35 86 E
SB 05	*P. rufipes*	S.I., Nelson, Matai Valley	41 19 31 89 S / 173 19 07 44 E
SB 26	*P. planus*	N.I., Auckland, Arataki, Waitakere Ra.	36 57 11 03 S / 174 35 35 31 E
SB 28	*P. reticulatus*	S. I.,Canterbury,Craigieburn, Mt. Ida	43 07 42 95 S / 171 44 26 75 E
SB 40	*P. reticulatus*	N.I., Ohakune,Visitor Ct., Mangawhero Forest Tr.	39 23 48 35 S / 175 25 14 65 E

(c) *Amychus*

Sample ID	Species	Location	Coordinates
CB 04	*A. manawatawhi*	N.I., Three Kings Is., Great Island, Tasman Va.	34 08 56 84 S / 172 08 59 91 E
CB 14.1	*A. candezei*	Ch. Is., South East Is.,Woolshed Bush	44 20 24 85 S / 176 10 18 05 W
CB 14.3	*A. candezei*	Ch. Is., South East Is.,Woolshed Bush	44 20 29 87 S / 176 10 29 06 W
CB 14.a/2	*A. candezei*	Ch. Is., South East Is.,Woolshed Bush	44 20 41 81 S / 176 10 20 81 W
CB 15	*A. candezei*	Ch. Is.,Main, Hapupu Reserve	43 48 03 42 S / 176 21 10 81 W
CB 16.1	*A. candezei*	Ch. Is.,The 44s	43 57 41 75 S / 175 49 27 01 W
CB 16.1/2	*A. candezei*	Ch. Is.,The 44s	43 57 41 75 S / 175 49 27 01 W
CB 16.3	*A. candezei*	Ch. Is.,The 44s	43 58 05 60 S / 175 49 23 30 W
CB 16.4	*A. candezei*	Ch. Is.,The 44s	43 57 40 54 S / 175 50 14 51 W
CB 16.a/2	*A. candezei*	Ch. Is.,The 44s	43 57 48 54 S / 175 49 54 44 W
CB 17.1	*A. candezei*	Ch. Is.,The Sisters,Middle Sister	43 34 10 00 S / 176 48 29 20 W
CB 17.2	*A. candezei*	Ch. Is.,The Sisters,Middle Sister	43 34 16 27 S / 176 48 34 66 W
CB 17.2a	*A. candezei*	Ch. Is.,The Sisters,Middle Sister	43 34 16 27 S / 176 48 34 66 W
CB 17a	*A. candezei*	Ch. Is.,The Sisters,Middle Sister	43 34 14 85 S / 176 48 42 06 W
CB 17a/2	*A. candezei*	Ch. Is.,The Sisters,Middle Sister	43 34 14 85 S / 176 48 42 06 W
CB 22	*A. granulatus*	S.I.,Cook Strait,Maud Is.,DoC house	41 05 36 59 S/ 173 54 27 05 E
CB 23	*A. granulatus*	S.I.,Cook Strait,Maud Is.,DoC house	41 05 36 59 S/ 173 54 27 05 E

(d) *Hadramphus & Lyperobius*

Sample ID	Species	Location	Coordinates
Wv 02	*H. stilbocarpae*	S.I.,Fiordland, Breaksea Is. headland	45 34 40 68S / 166 38 18 38E
Wv 03	*H. stilbocarpae*	S.I.,Fiordland, Breaksea Is. headland	45 34 35 85S / 166 38 20 94E
Wv 04	*H. stilbocarpae*	S.I.,Fiordland, South Breaksea Islet	45 34 02 38S / 166 38 19 29E
Wv 05	*H. stilbocarpae*	S.I.,Fiordland, South Breaksea Islet	45 34 58 26S / 166 38 32 18E
Wv 06	*H. spinipennis*	Ch. Is.,South East Island II	44 21 06 10S / 176 10 41 12W
Wv 07	*H. spinipennis*	Ch. Is.,South East Island II	44 21 06 10S / 176 10 41 12W
Wv 08	*H. spinipennis*	Ch. Is., South East Island	44 15 57 74S / 176 17 25 41W
Wv 09	*H. spinipennis*	Ch. Is.,Mangere Island patch	44 16 09 46S / 176 17 40 03W
Wv 10	*H. spinipennis*	Ch. Is.,Mangere Island patch	44 16 09 46S / 176 17 40 03W
Wv 11	*H. spinipennis*	Ch. Is.,South East Island I	44 21 10 71S / 176 10 00 07W
Wv 12	*H. spinipennis*	Ch. Is.,South East Island I	44 21 10 71S / 176 10 00 07W
Wv 13	*H. spinipennis*	Ch. Is.,Mangere Island patch	44 15 57 74S / 176 17 25 41W
Wv 14	*H. spinipennis*	Ch. Is., 28 Keefo van patch 3	44 15 51 33S / 176 17 41 74W
Wv 15	*H. spinipennis*	Ch. Is.,Mangere Island patch	44 16 32 22S / 176 18 05 54W
Wv 16	*H. spinipennis*	Ch. Is.,Mangere Island patch	44 16 32 22S / 176 18 05 54W
Wv 17	*H. spinipennis*	Ch. Is.,South East Island	44 20 46 90S / 176 10 18 50W
Wv 18	*H. tuberculatus*	S.I., Canterbury	43 15 33 67S / 171 39 44 76E
Wv 23	*H. stilbocarpae*	S.I., Fiordland, Secretary Is.	45 14 18 15S / 166 56 10 75E
Wv 24	*H. stilbocarpae*	S.I., Fiordland, Hawea Is.	45 35 28 59S / 166 38 38 86E
Wv 25	*H. stilbocarpae*	S.I., Fiordland, Puysegur Point	46 09 20 29S / 166 36 23 74E
Wv 26	*H. stilbocarpae*	S.I., Fiordland, Chalky Is.	46 03 10 79S / 166 30 39 18E
Wv 27	*H. stilbocarpae*	S.I., Fiordland, Wairaki Is.	45 35 31 38S / 166 38 10 93E
Wv 28	*H. stilbocarpae*	S.I.,Fiordland, Breaksea Is.	45 34 41 79S / 166 38 32 09E
Wv 31	*H. tuberculatus*	S.I., Canterbury	43 15 19 45S / 171 45 48 73E
LA31	*L. carinatus*	S.I., Mount Cook	43 15 25 45S / 170 45 48 73E
LHUT10	*L. huttoni*	S.I.,Kaikoura	42 15 35 13S / 173 36 26 64E
WvL.hut01	*L. huttoni*	N.I.,Wellington	41 19 42 91S / 174 42 37 78E
Wv20	*L. huttoni*	N.I., Wellington, Hawkins Hill	41 19 45 53S / 174 43 34 27E
WvAr45	*L. clarkei*	S.I.,Nelson, Mt. Arthur	41 14 52 57S / 172 36 55 06E
Ar410s	*L. clarkei*	S.I.,Nelson, Mt. Arthur	41 10 47 11S / 172 36 25 41E
Wv01	*L. hudsoni*	S.I.,Routeburn, Harris Saddle, Conical Hill	44 46 49 40S / 168 05 54 37E
WvL.hc2	*L. cupiendus*	S.I.,Otago, Carrick Range, Nevis Road	45 09 40 58S / 169 05 12 98E
WvL.hc3s	*L. cupiendus*	S.I.,Otago, Carrick Range, Nevis Road	45 10 50 14S / 169 03 10 80E
TAK60	*L. spedenii*	S.I.,Southland,Takitimu Mountains	45 40 57 97S / 167 53 01 45E

(e) *Anisolabis*

Sample ID	Species	Location	Coordinates
EW 01	*A. littorea*	N.I.,Wellington, The Sirens Rocks	41 20 53 24 S / 174 45 34 43 E
EW 02	*A. littorea*	Ch. Is.,Main, Long Beach South end	43 53 17 40 S / 176 32 38 62 W
EW 03	*A. littorea*	Ch. Is.,Main, The Crossing	43 52 25 34 S/ 176 31 16 34 W
EW 04	*A. littorea*	N.I., Manawatu. Tangimoana	40 18 32 87 S / 175 13 30 62 E
EW 05	*A. littorea*	N.I.,Bay of Plenty, Papamoa, Tauranga	37 61 56 59 S / 176 17 40 48 E
EW 09.1	*A. littorea*	S.I., Punakaiki, Pahautane Bay	42 08 02 11 S / 171 19 38 60 E

EW 15	*A. littorea*	N.I.,BOP, Ohope Beach,West End	37 58 51 44 S / 177 06 57 28 E
EW 16	*A. littorea*	N.I.,Coromandel,Whitianga,Buffalo Beach	36 48 27 87 S / 175 42 06 24 E
EW 17	*A. littorea*	N.I.,Taranaki,Ohawe Beach	39 34 41 13 S / 174 06 33 21 E
EW 19.1	*A. littorea*	Ch. Is.,Mangere Is.,Robin Bush	44 16 27 56 S / 176 18 03 46 W
EW 21	*A. littorea*	Ch. Is.,Main,Waitangi,Maipito Rd.	43 57 09 85 S / 176 33 17 85 W
EW 23.1	*A. littorea*	Ch. Is.,Main,Hapupu Reserve	43 48 08 68 S /176 20 59 74 W
EW 25.1	*A. littorea*	Ch. Is.,Main,Ohira Bay, Basalt Columns	43 48 21 38 S / 176 38 59 83 W
EW 27.1	*A. littorea*	Ch. Is.,The 44‘s	43 57 54 30 S / 175 49 48 88 W
EW 27.2	*A. littorea*	Ch. Is.,The 44‘s	43 57 54 30 S / 175 49 48 88 W
EW 28.1	*A. littorea*	Ch. Is.,The Sisters,Middle Sister	43 34 14 34 S / 176 48 39 60 W
EW 28.2	*A. littorea*	Ch. Is.,The Sisters,Middle Sister	43 34 14 34 S / 176 48 39 60 W
EW 30.2	*A. littorea*	S.I., Westland, Haast, Shipwreck Beach	43 46 23 78 S / 169 07 56 91 E
EW 34	*A. littorea*	N.I., Manawatu, Foxton Beach	40 29 31 67 S / 175 12 30 17 E
EW 35	*A. littorea*	S.I., Westland, Hokitika Beach	42 42 09 55 S / 170 58 42 60 E
EW 38	*A. littorea*	N.I., Taranaki, New Plymouth	39 02 27 37 S / 174 06 06 40 E
EW 40	*A. littorea*	N.I., Manawatu, Levin	40 35 59 35 S / 175 10 25 70 E
EW 41.1	*A. littorea*	S.I., Seaward Kaikoura, Marfell Beach	42 20 36 75 S /173 42 34 31 E
EW 42	*A. littorea*	S.I., Able Tasman, Awaroa	40 51 34 32 S / 173 02 00 21 E
EW 39	*A. kaspar*	N.I., Three Kings Islands	34 09 25 24 S / 172 08 10 20 E
EW 06.1	*A. occidentalis*	N.I.,Hawkes Bay, Ngarururo River mouth	39 33 56 85 S / 176 55 38 76 E
EW 29.1	*A. occidentalis*	N.I., Hawkes Bay, Ocean Beach	39 44 30 30 S / 177 00 44 79 E
EW 07.1	*Labidura riparia truncata*	N.I., Manawatu, Tangimoana Beach	outgroup
EW 08.1	*Labidura riparia truncata*	N.I.,Hawkes Bay, Napier, Ngarururo River	outgroup

Ch.Is. = Chatham Islands; N.I. = North Island, New Zealand; S.I. = South Island, New Zealand;

Table S6.2: Table of primers designed for and used in this study to amplify the COI fragment in *Anisolabis kaspar*.

Primer name	Sequence	Gene
EW_114R	3’ GTAGGTACAGCAATAATT	COI
EW_115F	5’ ATTATTGCTGTACCTACMG	COI
EW_322R	3’ AKACTGCTCCTATAGAAAGAAC	COI
EW_293F	5’ CTTATTATGTTGTWGCTCAC	COI
EW_514R	3’ CAACAWATATAAGCATCAGG	COI
EW_489F	5’ GATACCTCGWCGATAYTCAG	COI
EW_679R	3’ CTATGRTCTGMTGGTGGA	COI

VII
Conclusion

Biogeographers have long debated the origins and evolutionary history of the biota of New Zealand and questioned whether its characteristic biota is the product of long isolation or if diversification and colonization postdate this event (Pole, 1994; McGlone, 2005; McDowall, 2008). Many molecular studies of the plants and animals of New Zealand have in recent years been carried out focussing on speciation and colonization of the biota of New Zealand (e.g. Gillespie & Roderick, 2002; Sanmartin & Ronquist, 2004; Cook & Crisp, 2005; Knapp *et al.*, 2005) to try to answer the question of to what extent the biota is the product of dispersal events or can be seen as a vicariant, isolated biota (long isolation since the break-up of Gondwanaland). Based on these studies it has become apparent that the present biota of New Zealand is mostly the product of colonization and diversification, considerably postdating the break-up of Gondwanaland, and much more geologically recent (Goldberg *et al.*, 2008; Wallis & Trewick, 2009). New Zealand is nevertheless a very interesting system to study these sorts of issues because it has characteristics of both a continent and an island (Daugherty, 1993). Assumptions about the development of the biota cannot be made purely on the basis of geological scenarios, as has been the case for some truly oceanic islands such as Hawaii. Although some biogeographers have until recently pursued a biogeographic programme centred on the belief that New Zealand is an ancient land and its biota is thus ancient too, New Zealand's turbulent geological history is too complex and in some respects uncertain (Campbell & Hutching, 2007). A major advance in understanding is starting to pervade the biological community in recognition that the geological history of the Zealandian continent is in many respects quite distinct from the geological history of the New Zealand archipelago (Trewick et al. 2007, Campbell & Hutching, 2007). Whatever the fate of the biota that must have existed on Zealandia when it separated from Gondwanaland, the biology of New Zealand today reflects the geophysical history since the start of the Miocene. It remains unclear whether Zealandia was entirely submerged before the emergence of New Zealand, driven by tectonic activity on the Australian/Pacific plate boundary (~25 Ma), but the biological signature is that few lineages survived. Tectonic activity that began the formation of New Zealand resulted in movement of rock terraines and land areas with respect to one another, the formation of the Southern Alps and other mountain ranges, the submergence of some areas and considerable volcanic activity especially in North Island, which, together with climate cycling during the Pleistocene can be expected to have substantially influenced the countries biology. It is no surprise therefore that the nature of the New Zealand biota is complex and uniform patterns are few.

In order to properly understand the way the current biota could have evolved, we need to understand the fine scale processes that influence population structure and speciation. Thus, this thesis informs on the phylogeography and population genetic structure of a set of terrestrial

animal taxa in New Zealand and accompanying islands. Results indicate that much of the speciation and radiation in the examined taxa is very recent and cannot be explained by long isolation. Assumption about the age of New Zealand islands and thus their biota are found to be false, but patterns of speciation are shown to vary considerable among taxa and across space. In some groups, physical isolation appears to have been required to allow speciation (e.g. *Talitropsis* - chapter VI) with contiguous habitat occupied by a single species. Such cases very likely also indicate a role of extinction, loss of diversity in some regions, although identifying this directly is not possible. Other taxa (e.g. *Mecodema* beetles – chapter III) have responded quite differently to the same spatial and geophysical history; the huge diversity in New Zealand where most species have narrow spatial and habitat ranges might lead to the expectation that this genus would respond to dispersal to and colonisation of the Chatham Islands by prominent speciation, but this has not been the case. The same is true at a larger geographic scale; some lineages have close sister taxa in Australia and shallow genetic distances within and between species (e.g. *Phaulacridium* grasshoppers - chapter **V**) while others show deeper divergence to Australia, but relatively shallow speciation within New Zealand (*Mecodema* beetles - chapter III).

Analysis of relatively fine scale phylogeography also informs to the way inferences are made about the role of vicariant and dispersal events, and in particular to consider how extinction of lineages might lead to the false inference of ancient lineages. One example is the New Zealand woodpigeon, *Hemiphaga*, that exhibits low genetic divergence between populations in the New Zealand region including islands substantially distanced from one another, yet having deep phylogenetic divergence from its closest living relatives (chapter IV). Such a pattern is consistent with colonization in geologically recent times where it is accompanied by extinction of sister lineages elsewhere. At a fine scale, it is notable that, examination of taxa with prominent species diversity yields evidence of spatial and taxonomic monophyly and correlation of diversity with area, but taxon groups lacking species diversity reveal how this process most likely works. Even with mitochondrial data which suffers from having a small effective population size (i.e. understimating actual diversity), there is ample evidence (e.g. *Phaulacridium* - chapter V and *Anisolabis* - chapter VI) that colonisation of islands involves many individuals (and possibly sustained gene flow). This implies that within taxon diversity is lost over time after colonisation, rather than as a result of dispersal. Such an observation indicates a change in emphasis is needed in biogeography, away from considering dispersal (and its perceived rarity) to considering much more the factors that effect establishment, population expansion and adaptation.

Future work

There undoubtedly is the need for more molecular studies of the biota of New Zealand, to understand its origin and diversification patterns, as biogeographic interpretations based solely on observations of animal distributions can often be misleading. One important addition would be to include related taxa that are not from New Zealand into analyses, like Australia, New Caledonia and the Pacific, to advance the knowledge of the biotic relationships within the Australasian region and to be able to draw inferences on colonization events. This will also allow a comparative approach to phylogeogaphy as presented in this thesis. Furthermore it is important to better understand the mechanisms that drive dispersal and establishment of taxa and test for dispersal against vicariance scenarios in taxa with similar and different ecological traits, and to assess either congruence or incongruence of phylogeographic patterns in the biotic assemblage of these lineages. Additionally ecological and physiological studies could help to better understand the present biotic assemblage of New Zealand, as responses to geological and climatic factors play a major role in habitat occupancy, extinction, radiation and colonization of lineages. Further work should also focus on multi-gene approaches as different genes contain different information for phylogenetic reconstruction and timing of radiation or colonization events. Paradoxically, to better understand the historic processes that have governed the development of the extant biota, requires an improved understanding of populations, both in terms of their ecology and genetics. Understanding how populations respond to distant and changing opportunity will allow much better assessment of the likelihood of lineage loss over deeper time.

APPENDIX I

Geological Timescale

Geological time scale (from: New Zealand Geological Timescale, GNS science: Cooper *et al*., 2004). BP: before present; MYA: million years ago.

Period	Epoch	Unit	Start
Quarternary	Holocene		10,000 BP
	Pleistocene		1.81 MYA
Neogene	Pliocene		5.32 MYA
	Miocene	Late	11.2 MYA
		Middle	16.4 MYA
		Early	23.8 MYA
Paleogene	Oligocene	Late	28.5 MYA
		Early	33.7 MYA
	Eocene	Late	37.0 MYA
		Middle	49.0 MYA
		Early	55.5 MYA
	Paleocene	Late	61.0 MYA
		Early	65.0 MYA
Cretaceous		Late	99.6 MYA
		Early	145.5 MYA
Jurassic		Late	157.0 MYA
		Middle	175.6 MYA
		Early	199.6 MYA
Triassic		Late	237.0 MYA
		Middle	245.0 MYA
		Early	251.0 MYA

APPENDIX II

IUPAC code

A	Adenin
C	Cytosin
G	Guanin
T	Thymin (Uracil)
M	A or C
R	A or G
W	A or T (U)
S	C or G
Y	C or T (U)
K	G or T (U)
V	A or C or G
H	A or C or T (U)
D	A or G or T (U)
B	C or G or T (U)
N	A or C or G or T (U)

APPENDIX III

Software used in this study

ClustalX 1.81	ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/
T3 package	http://abacus.gene.ucl.ac.uk/
BioEdit	http://www.mbio.ncsu.edu/BioEdit/bioedit.html
DnaSP	http://www.ub.es/dnasp/
ClustalW	http://new.sh.mbl.edu/Course/Software/ClustalW/index.html
MacClade	http://phylogeny.arizona.edu/macclade/macclade.html
Mega	http://www.megasoftware.net/
Modeltest 3.7 & JModeltest	http://darwin.uvigo.es/software/modeltest.html
NCBI	http://www.ncbi.nlm.nih.gov/BLAST/
Paup 4.0b10	http://paup.csit.fsu.edu/
Se-Al	http://tree.bio.ed.ac.uk/software/seal/
Sequencher	http://www.genecodes.com/
TreeView 3.2	http://taxonomy.zoology.gla.ac.uk/rod/treeview.html
r8s 1.60	http://ginger.ucdavis.edu/r8s/
Oligo4	http://www.oligo.net/
PAML	http://abacus.gene.ucl.ac.uk/software/paml.html
TCS 1.21	http://darwin.uvigo.es/software/tcs.html
GeoDis 2.5	http://darwin.uvigo.es/software/geodis.html
Sequin	http://www.ncbi.nlm.nih.gov/Sequin/
BEAST	http://code.google.com/p/beast-mcmc/downloads/list
BEAUTi	http://code.google.com/p/beast-mcmc/downloads/list
LogCombiner	http://code.google.com/p/beast-mcmc/downloads/list
Tracer	http://tree.bio.ed.ac.uk/software/tracer/
TreeAnnotator	http://code.google.com/p/beast-mcmc/downloads/list
FigTree	http://tree.bio.ed.ac.uk/software/figtree/
Geneious	http://www.biomatters.com/installers/geneious/
MrBayes 3.1.2	http://mrbayes.csit.fsu.edu/
Arlequin 3.X	http://cmpg.unibe.ch/software/arlequin3/
Splitstree 4.X	wwww.splitstree.org
GoogleEarth	http://www.google.com/earth/index.html