School of Biological Sciences

**PHYLOGEOGRAPHY OF *MAORICICADA*
CAMPBELLI (HEMIPTERA: CICADIDAE):
MTDNA EVIDENCE INTERPRETED USING
PHYLOGENETICS AND
NESTED CLADE ANALYSIS**

by

Kathy Barbara Rose Hill

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ABSTRACT

New Zealand, because of its wide range of habitats and rapidly changing, well-studied palaeohistory provides an excellent opportunity to investigate the impact of earth processes on the distribution of taxa. There are now a variety of studies on plants that investigate such patterns, and a growing number of studies on animal taxa. Most studies have supported the hypothesis that current patterns of distribution of New Zealand taxa were created by dispersal, mediated by landscape and climatic conditions over time, and have contradicted the panbiogeographic hypothesis that explained distributions via vicariant landscape changes such as lateral displacement along the Alpine Fault. This thesis presents a detailed phylogeographic history of a widespread, endemic New Zealand cicada, *Maoricicada campbelli*, that is abundant throughout much of the South Island and is also found in the central volcanic plateau of the North Island. Mitochondrial DNA sequences of 223 individuals from 70 populations of *M. campbelli* were studied using both traditional phylogenetic methods and nested clade analysis (NCA). As found in a previous study of 35 *M. campbelli* individuals, geographic structuring was strong, with two main clades (North Island+northern South Island versus Otago) representing diverse lineages that may in fact be different species. Population structuring within the northern South Island clade suggested that the central South Island was mostly uninhabitable during glacial periods and demonstrated a sister-group relationship between northern and southern Southern Alps populations to the exclusion of the more central Southern Alps populations. Population histories estimated from NCA support the hypothesis that most *M.*

campbelli populations were formed through dispersal rather than via fragmentation or Alpine Fault vicariance. Three areas of ring-species-like secondary contact were found between the Otago and northern South Island clades, between lineages that had been isolated for approximately 2.3my. Further study is predicted to confirm the presence of additional suspected contact zones, and will demonstrate whether the different lineages are reproductively isolated or hybridising at these areas of secondary contact.

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Electronic Only Appendices (CD)

- Appendix 3.2 Total-mtDNA distance matrix
- Appendix 3.3 Total-mtDNA haplotype alignment
- Appendix 3.4 Haplotypes of all individuals sequenced
- Appendix 3.5 Total-mtDNA and COI Geodis input and output

The included CD contains all the text, tables, figures and appendices presented in this thesis, plus an additional nested clade map. Electronic nested clade figures are especially useful because readers can zoom-in on small details.

Table, figure and appendix legends presented in this thesis.

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Figure 3.6. Total-mtDNA nested clade geographic locations for South Island populations of *Maoricicada campbelli*. The location of all haplotypes and many higher clades are shown. Red with white internal are haplotypes, brown = 1-step clades, blue = 2-step clades, green = 3-step clades, orange = 4-step clades. All localities shown on these two pictures, except for the Otago clades, are joined into the same 5-step clade (5-1, northern-SI clade). Within text boxes white = Southern Alps A and C, blue = Southern Alps B, pink = Waitaki, yellow = NMK and Otago. Some geographical features mentioned in the text are indicated. DEM courtesy of GeographX, NZ.

Figure 4.1. Theoretical hypotheses of the distribution of *Maoricicada campbelli* over ca. 2.3my. Note that the present-day outline of New Zealand is used for all stages without reflecting known landscape changes over this time. Note also that clades are given their present-day range at all times. Ages of Otago and North Island clade separation events estimated by Buckley et al. (2001a). A dispersal scenario predicts that widespread *M. campbelli* were isolated in two refugia during the first glacial cycle, around 2.3mya. Note that the glacial refugium in the NMK area could have been in the North Island. The isolated Otago clade then remained static. The NMK clade dispersed to the North Island (or vice versa) approximately 0.9mya, then the NMK clade dispersed southward into the Waitaki and Southern Alps areas, coming into secondary contact with the Otago clade at the very end of this dispersal. A vicariance scenario predicts that a widespread *M. campbelli* species was subsequently fragmented into isolated populations evolving in situ. The Otago clade was the first to split off ca. 2.3mya, followed by the North Island clade ca. 0.9mya. The NMK clade split off, then lastly the Waitaki and Southern Alps clades separated. In the vicariance hypothesis, which requires the creation of barriers between populations, there would have been small scale dispersal around the edges to create secondary contact zones. Alternatively, population fragmentation could be postulated to follow a parapatric fragmentation model where a strong ecological gradient is coupled with assortative mating related to that gradient. In that case, contact zones would be primary. Inset: *M. campbelli* male.

Appendix 2.1 Additional information on molecular labwork methods.

Appendix 2.2. Map of part of insect mitochondrion. Locations of primers and areas sequenced for *Maoricicada campbelli* shown.

Appendix 3.1 Additional information on molecular labwork results.

Appendix 3.2 Alignment of 71 *Maoricicada campbelli* haplotypes and two outgroups (*Maoricicada clamitans* (M.clamit) and *Maoricicada phaeoptera* (M.phaeop) for 753bp of COI and 767bp of A6-A8. Blocks of haplotypes are shaded for ease of reading. The current gene is shown at the top of the page. Codons shaded black are those where the amino acid changes. The current number of bases is shown on the top right corner of each page. Note that codons in A8 are not aligned to spacing.

Appendix 3.3 *Maoricicada campbelli* mtDNA distance matrix for Total-mtDNA (COI+A6-A8) haplotypes.

Appendix 3.4 Sequenced individual *Maoricicada campbelli* COI, A6-A8 and Total-mtDNA haplotypes. Refer to Table 3.1 and Fig 3.1 for specific locality information.

Appendix 3.5 Geodis 2.0 input and output for COI and Total-mtDNA analyses of *Maoricicada campbelli* haplotypes. All output based on 10000 resamples.

1. INTRODUCTION

1.1 *Maoricicada campbelli*

A population study of the endemic New Zealand cicada *Maoricicada campbelli* is a good example of a natural experiment in speciation theory. This species has been divided into different populations that have been evolving and dispersing over the landscape for at least 2.3my, over and around mountains, valleys, rivers and plateaus (Buckley *et al.* 2001a) and eventually establishing secondary contact as shown here. Throughout this time, changes in New Zealand habitats have been brought about by plate tectonics, vulcanism and climatic cycles. *M. campbelli*, like all other *Maoricicada* species, is present only on the largest two of the main islands, although cicadas of other genera have managed to spread to almost all other temperate New Zealand islands. *M. campbelli* is the most widespread member of its genus in both habitat tolerance and altitudinal, latitudinal and longitudinal range. There are few studies of cicadas to date that directly document either individual or population movement (but see Cumber 1952, Fleming and Scott 1970, Williams and Simon 1995 and references therein, Hill *et al.* submitted) but the strong geographic structuring found in this genus suggests a lack of dispersal. *M. campbelli* is one of the smallest *Maoricicada* species (body size 13-17mm), and (like most other *Maoricicada* spp.) possesses short wings relative to body size, which may reduce dispersal capabilities relative to other cicadas. Like other members of the genus, *M. campbelli* is mostly found on stony outcroppings and river outwash, but is much more flexible than other species, being found on dry farm pasture and rocky mountaintop scree, as well as river edges and glacial moraines in what was probably ancestral habitat (Fleming 1971). Host plants

are currently unknown, but *M. campbelli* may oviposit and mature on a range of different plants that would allow occupation of their diverse habitats.

1.1.1 Prior *Maoricicada campbelli* analyses

Fleming (1971) suggested that *M. campbelli* evolved in an ice-age refugium in Otago and then expanded its range northwards, eventually dispersing to the central North Island volcanoes some 20,000 years ago. Buckley *et al.* (2001a) collected mitochondrial DNA (mtDNA) sequence information from 35 populations of *M. campbelli* (one individual per population) to test this idea. Their phylogenetic analyses separated these populations into five major clades and contradicted Fleming's hypothesis by suggesting that the current geographical range of *M. campbelli* is comprised of lineages that refuged in both northwest Nelson and Otago during Quaternary glacial maxima. Bayesian relaxed-clock dating estimated a divergence of 2.3 ± 0.5 my between the North Island+northern South Island and Otago populations (see Buckley *et al.* 2001a: Fig 1). They suggested that the most widespread clade dispersed south from the probable refugium in northwest Nelson, and currently surrounds the Otago clade on three sides. They hypothesised four contact zones but had direct evidence for none. Their molecular dating analysis suggested that the clade in the North Island was isolated for ca. 0.9my, managing to survive there throughout several glacial cycles.

1.1.2 New *Maoricicada campbelli* findings

Molecular phylogenetic evidence from 70 populations (223 individuals) of *Maoricicada campbelli* presented in this thesis supports the northwest Nelson

and Otago refuge hypothesis of Buckley *et al.* (2001a) for the initial ice-ages, and suggests that there were several refugia in the more recent glacial maxima. These new data also establish the existence of three of the predicted contact zones (Buckley *et al.* 2001a), and use nested clade analysis to suggest more specific hypotheses of population movement. These analyses suggest that populations of the northern South Island clade spread southward, creating secondary contact zones between the farthest south populations and the outer edges of the Otago clade (Figs 3.1, 3.6). This indicates secondary contact of a ring-like form, where the most divergent populations meet after moving around a barrier. In this case a barrier of unsuitable habitat was presumably created by early ice ages that eliminated the *M. campbelli* populations in the centre of the South Island that initially linked the northern South Island and Otago clades together. The repeated elimination of populations in the central South Island area also provides support for a glacial theory for the distribution of *M. campbelli* in the South Island.

1.2 New Zealand geography and palaeohistory

New Zealand lies at the boundary of the Pacific and Australian plates and today comprises three main islands, many small inshore islands and several offshore islands. New Zealand broke from Gondwanaland around 80mya and reached its present location approximately 65mya (Cooper and Millener 1993). Although aspects of the geological and biological history of New Zealand throughout the late Mesozoic and Cenozoic are still debated (Fleming 1980, Thornton 1994), the history of the last 5my is better known (McGlone 1988, McGlone *et al.* 2001). This period includes all of the ca. 18 Quaternary glacial-interglacial

cycles and most of the rapid mountain building of the Southern Alps. Although the Quaternary began 1.8mya, initial cooling began in the Pliocene approximately 2.5mya (Thornton 1994). The available terrestrial habitat of New Zealand changed with each glacial-interglacial cycle. The lowering of the sea level with glacial maxima increased the amount of coastal land and joined the three main islands. At the same time, the core habitable land was reduced by the formation of inhospitable glaciers along the Southern Alps and central North Island, surrounded by thousands of hectares of cold, dry tundra, plagued by gale force winds (Fleming 1980, Thornton 1994, Trewick and Wallace 2001). Since the start of the Kaikoura Orogeny around 25mya the Plates have slipped laterally, causing displacement along the Southern Alps of roughly 480km and separating the Nelson and Otago areas that once lay adjacent (Sutherland 1994, 1999). The most intense period of mountain building in the Southern Alps occurred in the centre during the last 5-6my (Fleming 1980, Batt *et al.* 2000, Chamberlain and Poage 2000). McGlone *et al.* (2001) describe this central Southern Alps area as a single alpine zone in relation to its flora, and note that there are no valleys descending below tree line for a ca. 200km stretch.

1.3 Hypotheses on the distribution of taxa throughout New Zealand

Two models have been proposed for the distribution of biota throughout New Zealand: a vicariance approach that suggests that taxa were moved around passively through the formation and reduction of barriers, such as tectonic movement of the landscape; and a dispersal approach that suggests that taxa moved themselves, mediated by climatic, volcanic and tectonic events. In the

central Southern Alps biotic gap zone, panbiogeographers suggest that lateral movement along the Alpine Fault resulted in this gap (e.g., Craw 1988, 1989, Heads 1998), whereas other biologists propose a glacial theory, whereby taxa were eliminated from this area by severe glaciers and unstable glacial outwash during ice-ages. Detailed studies of population movement are needed to test these hypotheses.

1.4 Nested clade analysis

Nested clade analysis (NCA) has been most effectively used to estimate the geographic history of populations within species, working best on widely distributed species with somewhat limited dispersal and only small amounts of genetic diversity (Templeton *et al.* 1987). Using sequences or restriction enzyme data, haplotypes are assembled into a cladistic network. These linked haplotypes are then grouped into clades, starting with haplotypes only one mutation apart, then moving to larger clades encompassing more and more haplotypes. These “nested clades” are then analysed using geographic distances within and between clades at different levels to estimate the evolutionary history of movement of the haplotypes to their present populations. The previous study (Buckley *et al.* 2001a) indicated that *M. campbelli* should be an ideal candidate for nested clade analysis.

1.5 Previous phylogeographical studies of animal taxa in New Zealand

Studies employing molecular methods to explore the phylogenetic and phylogeographic structure of New Zealand taxa are becoming more and more detailed and this study on *M. campbelli* represents one of the most detailed

molecular studies of a single animal taxon within New Zealand. Trewick *et al.* (2000) studied the mtDNA of 21 populations of a species of alpine scree weta (*Deinacrida connectens*) that is found mostly at higher altitudes than *M. campbelli*. As found in *M. campbelli* (Buckley *et al.* 2001a and this study), populations of *D. connectens* from different areas showed much higher genetic divergence than that normally reported for intraspecific variation, however their estimated radiation time of about 4mya ties population differentiation to mountain building rather than climate effects from glacial cycles. Trewick *et al.* (2000) found 36 different haplotypes, with the deepest genetic divergence found between populations that were also the most divergent geographically (Nelson and Southland). This scree weta is currently a high alpine taxon, and Trewick *et al.* (2000) suggest that the range of *D. connectens* is actually restricted during interglacials, and wider during glacial periods. Chin and Gemmel (2004) studied species of South Island cockroaches in the genus *Celatoblatta*. Their taxa show results somewhat consistent with the *Maoricicada* as a whole (Buckley *et al.* 2001b), with divergences between taxa around 4.2mya explained by central Southern Alps mountain building. One species (*C. hesperia*), however, is found on both sides of the Southern Alps, with dating suggesting populations diverged during glacial cycles. The two *Celatoblatta* species found within the area of the *M. campbelli* Otago clade area were in one divergent group, however there was little sampling of this area. Chin and Gemmel (2004) also describe potential secondary contact and hybridisation within the central South Island biotic gap area (see Fig 3.1B), between species found north, south and centrally, and they suggest that the number of different haplotypes in this central area shows *Celatoblatta* survived there during glacial

maxima. The phylogeography of the forest-restricted New Zealand short-tailed bat, *Mystacina tuberculata*, was studied using mtDNA control region sequences by Lloyd (2003). Lloyd analysed 241 individuals from 13 populations using various methods including nested clade analysis. Unfortunately for comparison with *M. campbelli*, most short-tailed bat populations are in the North Island, however the two South Island populations were unique. Hypotheses on the phylogeography of the South Island populations of the short-tailed bat suggested that they were probably isolated in a glacial refuge in the north of the South Island and then spread down either side of the Southern Alps during interglacials. Lloyd (2003) suggested that areas of the central North Island and most of the South Island experienced repeated introductions and extinctions throughout the glacial cycling. Interestingly, although *Nothofagus* existed south of the biotic gap area during at least the last glacial maximum, Lloyd (2003) argues that the climate in this area would have been too severe for populations of short-tailed bat to have survived.

Each of these papers demonstrates geographical structuring within animal taxa due to climatic or geological events, and in some cases with contact or hybridisation zones suggested, and potential cryptic species discovered. This study of *M. campbelli* also sought to understand population structuring within a widespread taxon; whether populations moved through vicariance or dispersal techniques; if there currently were or had been contact zones between populations and whether any populations had diverged enough to actually be cryptic species.

2. METHODS

2.1 Collecting

Maoricicada campbelli males and females were collected from a range of localities throughout New Zealand. Collection sites were identified from historical records, a knowledge of habitat or from hearing the songs of the males. Individuals were captured by hand or with a net; males were located by sound, females were noticed incidentally in the course of collecting the males. Specimens were stored in individually labeled containers in 95% EtOH at -4°C until extraction was completed. They were then stored long-term at -80°C. The precise location of populations sampled was determined by using GPS in the field, or (for older samples) by extrapolating from high-definition maps if the GPS was unavailable. The GPS data were entered into ArcView (GIS software, ESRI) to error check the location of sites and to output site maps, and was used in the NCAs to calculate geographical distances.

Voucher specimens will be deposited in the New Zealand Arthropod Collection (NZAC), the Museum of New Zealand Te Papa Tongarewa collection and at the University of Connecticut.

2.2 Labwork: extraction, amplification and sequencing of mtDNA

2.2.1 Genomic DNA extraction

DNA was extracted from approximately 0.1g of thorax muscle tissue using a Qiagen DNeasy™ Tissue Kit following the manufacturers instructions. The final elution was made into two 200µL aliquots of ddH₂O, which were stored

separately at 4°C. When the work was completed, extractions were stored at -80°C for future use. Additional information on extraction methods trialled can be found in Appendix 2.1.

2.2.2 PCR amplification and purification

A polymerase chain reaction (PCR) method was used to amplify two portions of the mtDNA genome: approximately 800 bp of the 3' end of the Cytochrome Oxidase subunit I (COI) gene was amplified using the conservative primers C1-J-2195 and TL2-N-3014 (Simon *et al.* 1994); and approximately 800 bp of a gene portion spanning the A6-A8 region (part of tRNA^{Lys} (tP), tRNA^{Asp} (tK), ATPase subunit 8 (A8) and most of ATPase subunit 6 (A6)) was amplified using the primers TK-J-3799 and A6-N-4570 (Buckley *et al.* 2001a). Additional primers were constructed using Oligo 5 (primer analysis software, Molecular Biology Insights (MBI)) and named according to the convention set out in Simon *et al.* 1994. A second primer was constructed for the 3' end of the A6-A8 region from COIII sequence obtained by Thomas Buckley (unpublished data) and was used to amplify the A6-A8 region with problematic sequences. This primer (sequence 5'-ATACCAGATACAAAAGTTAGAGC-3') was located near the 5' end of the COIII region and was named C3-N-4834. Internal primers were constructed to sequence the ends of the COI fragment in cases where base calls were disputed near the beginning or end of the sequence fragment, or when nuclear copies of mitochondrial genes (numts) were suspected. These internal primers were constructed from *M. campbelli* COI sequence data and named C1-J-2615 (sequence 5'-GCTATTATGGCAAGATTTATTCA-3') and C1-N-2576 (sequence 5'-TAATGAAAATGAGCAACTAC-3').

The COI gene regions (whole and internal) were amplified using Qiagen Taq DNA Polymerase enzyme. The 25µl reaction consisted of 2µl of genomic DNA added to a 23µl reaction mix (10.35µl ddH₂O; 5.0µl Qiagen PCR solution; 2.5µl Qiagen *10 PCR solution; 2µl of each primer; 1µl dNTP; 0.15µl Qiagen Taq enzyme), covered with 20µl mineral oil and cycled in a Perkin Elmer Thermal Cycler. Cycle sequence: initial: 94°C for 2 mins; then 30 cycles of: 94°C for 45 secs; 56°C for 45secs; 72°C for 1min 15secs; final extension: 72°C for 10 mins.

The A6-A8 gene region was amplified using either of two methods that worked equally well. 1) 1.5µl genomic DNA from extraction elution one was added to a 23.5µl PCR reaction mix using Amplitaq Gold enzyme and solutions (14.42µl ddH₂O; 2.4µl Amplitaq Gold PCR solution; 2.4µl Amplitaq gold MgCl solution; 1.0µl each primer; 2.0µl dNTP; 0.28µl Amplitaq Gold Taq enzyme) and run on a MJ Research Gradient PCR machine. Cycle sequence: initial: 94°C for 2 mins; then 30 cycles of: 94°C for 45secs; 63°C for 45secs; 72°C for 1min 15secs; final extension: 72°C for 10 mins. 2) 2µl genomic DNA from extraction elution one was added to a 23µl PCR reaction mix using Amplitaq Gold master mix (12.5µl Amplitaq Gold master mix; 7.5µl ddH₂O; 1.5µl each primer) and run on a 2700 Thermal Cycler using the two-step method: initial :94°C for 10 mins; then 30 cycles of 94°C for 15 secs; 64°C for 1 min; final extension: 72°C for 7 mins. A 12.5µl PCR reaction mix reduced proportionally and used for the last A6-A8 amplifications worked equally well or better. Additional information on PCR amplification can be found in Appendix 2.1.

PCR products were purified using the Roche Applied Science High Pure PCR Product Purification Kit following the manufacturers instructions and eluted into 8-20µl elution buffer.

2.2.3 Sequencing and alignment

Each specimen was sequenced in both directions for the COI gene region, and selected individuals were sequenced in both directions for the A6-A8 gene regions using a standard PCR cycle sequencing mixture (Bigdye version 2) and reaction, and then sequenced on a 377 Perkin Elmer automated sequencer with ABI Prism Sequence Analysis 3.4.1 software. Individuals with unreadable sequences, either from amplification/sequencing errors or sequences containing suspected numts were resequenced or sequenced with the internal (COI) or external (A6-A8) primers. Sequences that still proved unreadable were discarded. Additional information on obtaining readable sequence can be found in Appendix 2.1.

Sequences obtained by Buckley *et al.* (2001a) were used as templates for sequence alignment. DNASTar software was used to align the sequences, which were edited, translated, and checked manually. The forward and reverse for each individual *M. campbelli* COI and A6-A8 mtDNA region were aligned and checked, and then all sequences for each mtDNA region were aligned and assigned to specific haplotypes.

2.3 Analyses

2.3.1 Analyses: phylogenetic methods

Phylogenetic analyses were performed using maximum likelihood (ML, Felsenstein 1981) implemented in PAUP* 4.01 (Swofford 1998) and Bayesian MCMC analyses using MrBayes 3.04b (Huelsenbeck and Ronquist 2001). All phylogenetic trees were rooted using the congeneric species *M. clamitans* and *M. phaeoptera* as in Buckley *et al.* (2001a). Base composition stationarity tests were run in PAUP* 4.01.

2.3.1.1 Maximum likelihood analyses

ML analyses were performed on the Total mtDNA (COI+tRNA^{Asp}+A6+A8) haplotype dataset. Modeltest (Posada and Crandall 1998) was used to choose the model of evolution (TrN+I) for the ML analysis. TrN is a modification of the GTR model, where only transitions (G-A, T-C) are estimated from the data and the other four rates are given as one. A heuristic search was completed using empirical base frequencies and other parameters estimated from Modeltest (G-A= 27.543200, T-C= 20.065200, I=0.7833), with the initial trees obtained via random stepwise addition followed by TBR branch swapping for 10 replicates. Statistical support for nodes was estimated using the nonparametric bootstrap (Felsenstein 1985), which was run using these same parameters for 44 pseudoreplicates. The large data set with its very closely related taxa meant that analyses were slow, hence the number of replicates was low.

2.3.1.2 Bayesian analyses

The method of Frati *et al.* (1997) was used to choose the models for each partition used in Bayesian analyses. The model-testing method of Frati *et al.* (1997), like Modeltest, uses an algorithm to test whether one model is better than another, but unlike Modeltest starts with the most complex model and then tests it against nested models until it finds a significant difference in likelihood. The Akaike Information Criterion (AIC) (Akaike 1973, 1974) was also used in conjunction with this method to find the best model. The selected model is not significantly different in likelihood from the most parameter-rich model but itself has as few parameters as possible. Bayesian analyses were performed on a partitioned Total-mtDNA dataset and run for 4 million generations, sampling every 100 generations. There is no consensus on how to design data partitions for the best result. Adding parameters usually results in a higher likelihood, however using too many partitions can result in overparameterisation which causes a loss of power in the analysis (Lemmon and Moriarty 2004). A conservative model using three data partitions was chosen: coding DNA was split into 1st+2nd base pairs modelled with HKY85+I and 3rd base pairs modelled with GTR+ Γ , non-coding DNA (tRNA^{Asp}) was modelled with HKY85+I. The program Tracer 1.1 (Andrew Rambaut and Alexei Drummond, University of Oxford, UK) was used to test the Bayesian datasets for stationarity and parameter autocorrelation. Branch support was estimated using Bayesian posterior probabilities.

2.3.2 Analyses: phylogeographic methods (nested clade analysis)

2.3.2.1 nested clade analysis network design

Networks for COI, tRNA^{Asp}, A6, A8, and Total mtDNA (COI+tRNA^{Asp}+A6+A8) haplotypes were constructed using the computer program TCS 1.13 (software for estimating gene genealogies, Clement *et al.* 2000). An unrooted network linking all haplotypes through a coalescent method that estimates the 95% probability of haplotypes being linked together parsimoniously through ancestral states was created (Templeton *et al.* 1992). The haplotypes within these maximum-step clades were joined in a network of steps, with a single mutation being one step. Inferred haplotypes were created to provide links between haplotypes more than one mutational step away. The haplotype networks were then grouped into nested clades using the method of Templeton *et al.* (1987). Proceeding always from the tips of the network tree, these tip clades were grouped with clades one mutational step away into a higher nesting clade, and then the next most internal clades grouped together, until all the clades were grouped into a higher nesting level. These clades were then grouped into higher clades, and so on, stopping at the last clade-step before the entire network would be encompassed by one clade. Ambiguities formed from loops in the network and odd numbers of clades were dealt with using coalescent theory (Crandall and Templeton 1993, Templeton and Sing 1993). Coalescent theory is used to break loops and to assign odd numbers of clades into nesting-clades by a set of rules that assume that clades with more individuals are likely to be ancestral, and clades with few individuals (especially singletons (clades with a single individual)) are likely to be new mutations. Odd numbers of clades

are grouped so that the odd clade is grouped with the clade with the least number of sampled individuals to increase the power in the geographical associations analysis.

2.3.2.2 rooting the nested clade analysis network

To root the *M. campbelli* nested clade diagrams we analysed all the individual sequences for COI and Total-mtDNA using TCS, instead of limiting the data to haplotypes. This outputs the probability for each haplotype that it is the root of the cladogram, worked out by considering the frequency of each haplotype and its position within the cladogram (see Castelleo and Templeton 1994). For the Total mtDNA cladogram we also viewed these outgroup weights in combination with the geographical coalescent method described in Crandall and Templeton (1993), which considers the size of the geographical area that each haplotype was found over. We then compared the root suggested by NCA to the root suggested by phylogenetic analysis.

2.3.2.3 nested clade analysis using geographical associations

The program Geodis 2.0 (Posada *et al.*, 2000) was used to analyse clade structure for geographical associations. The latitude and longitude were entered for each population and Geodis performed the calculations for distances within and between clades. The Geodis output was used in conjunction with the inference key (Nov 2001 version) provided with the program to estimate the history of population movement. A new key (25 Nov 2003: Templeton 2004) was also used to determine if there were any differences in the resulting population hypotheses. Clades were plotted onto a

map of New Zealand for visual geographical analyses

3. RESULTS

3.1 Collecting

Maoricicada campbelli were collected from 70 populations throughout New Zealand (Table 3.1). Specimens were individually labelled with a 9-digit series: the last two digits of the year, the 2-letter standardised New Zealand district code from Crosby *et al.* (1998) a unique, usually informative, three-letter site code and a two-digit specimen code. For example, 02.MK.LOH.01 was the first specimen collected from the lower end of the ski road at Lake Ohau in the Mackenzie district in 2002. This allowed the use of the same code throughout the work, ease of identifying specimens from the same districts, years and sites, and minimised labelling errors. A two-letter country code (NZ) preceding the district code was also recorded, but is not necessary in this publication. In Table 3.1, districts are sorted roughly north-south (for district boundaries see Crosby *et al.*, 1998). Populations are listed alphabetically within districts, and the GPS is the center of all individuals collected within one km of contiguous habitat. Only three of the 70 populations (MK.LOH, WD.HAR, CO.CRA) grouped individuals that were not collected from exactly the same place, and all were no greater than 1km of contiguous habitat apart, and shared the same or similar haplotypes. Almost all individuals collected were males, females (identified in Appendix 3.4) were very difficult to find. *M. campbelli* were usually very abundant where found, however collecting conditions were not always conducive and populations collected in bad weather were represented by one or very few individuals. Male *M. campbelli* were usually easy to collect when

singing, as they could be attracted to the collector by simulating the female response (wingflicks) to the male's song by snapping the fingers or clicking the tongue lightly. In bad weather *M. campbelli* were almost impossible to find, as they were well camouflaged to their rocky habitat and the males did not sing. One *M. campbelli* was collected in the rain by turning over rocks in a likely area.

Most of the *M. campbelli* populations that were additional to the sites of Buckley *et al.* (2001a) filled in gaps, rather than extending the sampled range of the species. The range of some clades, however, was extended by the sequencing of multiple individuals from some populations. No distant populations of *M. campbelli* were found in the North Island. Historical records from near Wellington remain undiscovered and represent either unfound or extinct populations, or possibly incorrect records of a similar species. The oral record listed in Fleming 1971 from Mt Tauhara, north of Mt Ruapehu, was most probably an incorrect report of the congeneric species *Maoricicada iolanthe*, which has an almost identical song and had not been reported from that location. In December 2001 we found only *M. iolanthe* from the base to the top of the mountain, however it is still possible that *M. campbelli* could be found in this location in later months. Further searching is needed to determine whether *M. campbelli* is definitely present or absent from Mt Tauhara. Previously unsampled populations in northwest Nelson and southern Otago were collected, which extended the sampled range of the species. Several interesting areas in the South Island containing *M. campbelli* were sampled after completion of these analyses, and will be used in further phylogenetic studies.

Table 3.1 *Maoricicada campbelli* collecting localities (70 populations), GPS coordinates and haplotypes found in each population. Locations are sorted roughly north-south by district. District boundaries follow Crosby *et al.* 1998.

Code	†2001 population number	Location	††GPS coordinates	COI	†††mtDNA haplotypes A6-A8	Total-mtDNA
Taupo						
TO.BRR		SH48 S of Whakapapa, W side of Mt Ruapehu	(39°10.60'S, 175°30.75'E)	A1 (2)	a1 (1)	A1a1 (1)
TO.MAN		End of Mangatepopo road, W side of Mt Ruapehu	(39°08.78'S, 175°34.85'E)	A1 (3)	a1 (1)	A1a1 (1)
TO.TSR		Tukino Skifield Road, off SH1, E side of Mt Ruapehu	(39°17.78'S, 175°44.13'E)	A1 (3)	a1 (2)	A1a1 (1)
				A3 (1)	a2 (1)	A3a1 (1)
				A4 (1)		A4a2 (1)
TO.WSF	[15]	Whakapapa Ski Field, W side of Mt Ruapehu	(39°13.85'S, 175°33.60'E)	A2 (1)	a1 (1)	A2a1 (1)
Marlborough						
MB.KOW		Kowhai Scenic Reserve, off SH63, S side of Wairau R.	(41°42.84'S, 173°06.76'E)	BM2 (1)	bm1 (1)	BM2bm1 (1)
MB.KSR	[31]	Kowhai Scenic Reserve, N side of Wairau River	(41°36.00'S, 173°22.20'E)	BM1 (1)	bm3 (1)	BM1bm3 (1)
MB.LSG	[36]	Lake Sedgemere, off Rainbow-Wairau Divide Rd,	(42°08.20'S, 172°54.75'E)	BM1 (1)	bm2 (1)	BM1bm2 (1)
MB.LTT	[41]	S tip of Lake Tennyson, off Rainbow-Wairau Divide Rd	(42°12.80'S, 172°44.40'E)	BSA6 (1)	bsa6 (1)	BSA6bsa6 (1)
				BSA7 (1)	bsa11 (1)	BSA7bsa11
(1)						
MB.NST	[42]	Nocatchem Stream, Rainbow-Wairau Divide Rd	(41°48.90'S, 172°54.85'E)	BM1 (1)	bm1 (2)	BM1bm1 (1)
				BM2 (1)	bm5 (1)	BM2bm5 (1)
				BM3 (1)		BM3bm1 (1)
MB.PVS	[13]	Pine Valley Stream, N side of Wairau river	(41°32.17'S, 173°32.59'E)	BM2 (1)	bm1 (2)	BM2bm1 (1)
				BM4 (1)		BM4bm1 (1)
MB.RFT		Rag & Famish Creek, Rainbow-Wairau Divide Rd	(42°08.78'S, 172°51.08'E)	BM5 (1)	bm2 (1)	BM5bm2 (1)
MB.SRW	[35]	Serpentine Creek, Rainbow-Wairau Divide Rd	(42°11.14'S, 172°46.82'E)	BSA1 (1)	bsa1 (1)	BSA1bsa1 (1)
MB.WRR		E of St Arnaud on SH63, just across Wairau River	(41°43.21'S, 173°05.75'E)	BM1 (1)	bm1 (1)	BM1bm4 (1)
				BM3 (1)	bm4 (1)	BM3bm1 (1)
				BM6 (1)	bm6 (1)	BM6bm6 (1)
Nelson						
NN.COB		Cobb Reservoir, by dam	(41°06.35'S 172°41.20'E)	BN1 (3)	bn1 (4)	BN1bn1 (2)
				BN2 (1)	bn2 (1)	BN1bn2 (1)
				BN3 (1)		BN2bn1 (1)
						BN3bn1 (1)
Kaikoura						
KA.ORO	[10]	Oaro River, off SH1, S of Kaikoura	(42°30.20'S, 173°29.15'E)	BK1 (1)	bk1 (1)	BK1bk1 (1)
KA.WAN	[11]	Wandle River, SH70, N of Waiau	(42°30.56'S, 173°08.42'E)	BK2 (1)	bk2 (1)	BK2bk2 (1)

Code	†2001 population number	Location	††GPS coordinates	†††mtDNA haplotypes COI	A6-A8	Total-mtDNA
Buller						
BR.BRD		SH65, approx 5km W of Lewis Pass	(42°22 00'S, 172°21 00'E)	BSA1 (1)	<i>bsa5</i> (1)	BSA1 <i>bsa5</i> (1)
BR.BUR		Warwick River, SH65, S of Burnbrae	(42°04 93'S, 172°14 24'E)	BSA1 (1)	<i>bsa1</i> (1)	BSA1 <i>bsa2</i> (1)
				BSA3 (1)	<i>bsa2</i> (1)	BSA3 <i>bsa1</i> (1)
BR.STJ	[14]	Start of St James Walkway, Lewis Pass, off SH7	(42°21 85'S, 172°24 00'E)	BSA1 (1)	<i>bsa1</i> (2)	BSA1 <i>bsa1</i> (1)
				BSA5 (1)		BSA5 <i>bsa1</i> (1)
North Canterbury						
NC.ANP		Waimakariri River, W of Bealey, off SH73	(43 01 58'S, 171 37 47'E)	BSA1 (3)	<i>bsa1</i> (3)	BSA1 <i>bsa1</i> (1)
				BSA4 (1)	<i>bsa7</i> (1)	BSA4 <i>bsa1</i> (1)
				BSA8 (1)	<i>bsb3</i> (1)	BSA8 <i>bsa1</i> (1)
				BSB1 (1)		BSB1 <i>bsb3</i> (1)
				BSB5 (1)		BSB5 <i>bsa7</i> (1)
NC.EGW	[18]	Waiau River, E of Glynn Wye, Lewis Pass	(42°34 90'S, 172°35 20'E)	BSA1 (1)	<i>bsa1</i> (1)	BSA1 <i>bsa1</i> (1)
NC.SBH	[21]	Hurunui River, S Branch	(42°46 35'S, 172°10 90'E)	BSA1 (1)	<i>bsa1</i> (1)	BSA1 <i>bsa1</i> (1)
NC.WGW	[16]	Hope/Waiau River, W of Glynn Wye, Lewis Pass	(42°34 60'S, 172°27 75'E)	BSA1 (1)	<i>bsa1</i> (1)	BSA1 <i>bsa1</i> (1)
Mid Canterbury						
MC.CSR		Craieburn Skifield Road, off SH73	(43°09 16'S, 171°44 25'E)	BSA1 (1)	<i>bsa1</i> (2)	BSA1 <i>bsa1</i> (1)
				BSA2 (1)		BSA2 <i>bsa1</i> (1)
MC.PRT		Just S of Porter's Skifield Rd, SH73 W of Porter's Pass	(43°15 81'S 171°42 69'E)	BSA1 (4)	<i>bs1</i> (1)	BSA1 <i>bs1</i> (1)
MC.SCR		Just W of Porters Pass, SH73	(43°17 75'S 171°43 00'E)	BW9 (1)	<i>bw7</i> (1)	BW9 <i>bw7</i> (1)
South Canterbury						
SC.BWT		Bullock Wagon Trail, just off SH8, W of Burkes Pass	(44°05 91'S, 170°33 98'E)	BW3 (2)	<i>bw2</i> (2)	BW3 <i>bw2</i> (2)
Mackenzie						
MK.AHU	[44]	Ahuriri river, on Birchwood Station Road, NW of SH8	(44°28 79'S, 169°43 98'E)	CN1 (9)	<i>cn1</i> (7)	CN1 <i>cn1</i> (6)
				CS4 (1)		CS4 <i>cn1</i> (1)
MK.BCL	[33]	Boundary Stream, East side of Lake Tekapo	(43°54.86'S, 170°33 50'E)	BW1 (1)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.BNO		DoC trail to Ben Ohau, E side of Tay stream, off SH80	(44°09 81'S, 170°06 11'E)	BW1 (1)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.HVI		Hopkins River Valley N of Lake Ohau, 1.3km from gate	(44°04 80'S, 169°49 75'E)	BW1 (4)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.HVN		Hopkins River Valley N of Lake Ohau, 5.6km from gate	(44°03 30'S, 169°49 10'E)	BW1 (1)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.LIN	[28]	Lindis Pass rest area	(44°35 38'S, 169°38 60'E)	CN1 (3)	<i>cn1</i> (3)	CN1 <i>cn1</i> (3)
MK.LOH	[37]	Lower end of ski road to base at W Edge of Lake Ohau	(44°14.00'S, 169°49.00'E)	BW1 (8)	<i>bw1</i> (7)	BW1 <i>bw1</i> (5)
				BW2 (1)	<i>cn1</i> (6)	BW2 <i>bw1</i> (1)
				BW4 (1)	<i>cn3</i> (2)	BW3 <i>bw1</i> (1)
				CN1 (11)		CN1 <i>cn1</i> (4)
				CN2 (2)		CN1 <i>cn3</i> (2)
						CN2 <i>cn1</i> (2)

Code	†2001 population number	Location	††GPS coordinates	†††mtDNA haplotypes		
				COI	A6-A8	Total-mtDNA
Mackenzie continued						
MK.LTM		Tasman Glacier 4WD track, Mt Cook National Park	(43°39.61'S, 170°10.68'E)	BW1 (1)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.MCA	[23]	SH80 to Mt Cook, N of SH8, W side of Lake Pukaki	(44°06.00'S, 170°08.00'E)	BW1 (1)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.MCB	[27]	Birch Stream, Mt Cook Natl Park	(43°44.30'S, 170°04.45'E)	BW1(1)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.MCR		SH80 to Mt Cook, S of Airport, W side of Tasman River	(43°48.68'S, 170°06.72'E)	BW1 (1)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.MCV		Mt Cook Visitor's Centre	(43°44.11'S, 170°05.73'E)	BW1 (2)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.OHA		Near top of Lake Ohau Skifield Rd	(44°13.70'S, 169°46.85'E)	BW1 (1)	<i>bw1</i> (1)	BW1 <i>bw1</i> (1)
MK.RHD		Rhoborough Downs Rd at Twizel River, W of SH8	(44°11.83'S, 170°05.79'E)	CN1 (1)	<i>cn1</i> (1)	CN1 <i>cn1</i> (1)
				BW1 (11) BW6 (1)	<i>bw1</i> (3)	BW1 <i>bw1</i> (2) BW6 <i>bw1</i> (1)
Westland						
WD.ARP	[22]	Arthurs Pass, Arthurs Pass National Park	(42°54.60'S, 171°33.60'E)	BSA2 (1)	<i>bsa1</i> (1)	BSA2 <i>bsa1</i> (1)
WD.HAR	[7]	Haast River, SH6, Just N of Haast Pass	(44°05.90'S, 169°21.76'E)	BSC1 (2)	<i>bsa1</i> (4)	BSC1 <i>bsa1</i> (2)
				BSC4 (1)	<i>bsa10</i> (1)	BSC4 <i>bsa10</i>
				BSC5 (1) BSC6 (1)		BSC5 <i>bsa1</i> (1) BSC6 <i>bsa1</i> (1)
WD.OTR		E side of Otira river, at bridge, SH73	(42°51.20'S, 171°33.62'E)	BSA1 (2)	<i>bsa3</i> (1)	BSA1 <i>bsa3</i> (1)
WD.PLC	[40]	Peg Leg Creek, Arthurs Pass National Park	(42°53.79'S, 171°33.56'E)	BSA2 (1)	<i>bsa1</i> (1)	BSA2 <i>bsa1</i> (1)
Fiordland						
FD.BST	[43]	South of Boundary Creek, SH94, N of Te Anau	(45°18.30'S, 167°47.75'E)	BSC2 (1)	<i>bsa1</i> (1)	BSC2 <i>bsa1</i> (1)
FD.HCT	[30]	Henry's Creek campground, SH94, N of Te Anau	(45°14.40'S, 167°48.85'E)	BSC2 (2)	<i>bsa1</i> (1)	BSC2 <i>bsa1</i> (1)
FD.HME		E side of Homer Tunnel, SH94 S of Milford Sound	(44°45.97'S, 167°59.35'E)	BSC1 (2)	<i>bsa1</i> (1)	BSC1 <i>bsa1</i> (1)
Central Otago						
CO.ABT	[9]	Albert Town, SE of Lake Wanaka	(44°40.80'S, 169°11.50'E)	CN1 (1)	<i>cn2</i> (1)	CN1 <i>cn2</i> (1)
CO.AWR	[32]	Awakino Ski Field Road, off SH83 near Kurow	(44°45.79'S, 170°21.62'E)	BW1 (3)	<i>bw1</i> (3)	BW1 <i>bw3</i> (2)
				BW2 (1)	<i>bw3</i> (1)	BW2 <i>bw3</i> (1)
				BW7 (1)	<i>bw5</i> (1)	BW1 <i>bw5</i> (1)
				BW8 (1)	<i>bw6</i> (1)	BW7 <i>bw1</i> (1) BW8 <i>bw6</i> (1)
CO.CRA		Near Cardrona River, SH89, S of Cardrona	(44°54.22'S, 168°59.10'E)	CN1 (2)	<i>cn1</i> (1)	CN1 <i>cn1</i> (1)
				CN3 (1)	<i>cn2</i> (2);	CN3 <i>cn2</i> (1)
				CN4 (1)		CN4 <i>cn2</i> (1)
CO.KYE	[29]	Kye Burn River, S of Dansey's Pass	(45°00.23'S, 170°16.45'E)	BW5 (1)	<i>bw4</i> (1)	BW5 <i>bw4</i> (1)
CO.LTO	[38]	Little Omarama Stream, S of Omarama	(44°35.85'S, 169°52.80'E)	CN1 (1)	<i>cn1</i> (1)	CN1 <i>cn1</i> (1)
CO.OMR	[24]	Old Man Range, Symes Rd to Obelisk	(45°20.33'S, 196°15.26'E)	CN1 (2)	<i>cn1</i> (1)	CN1 <i>cn1</i> (1)
				CS3 (1)	<i>cs1</i> (1)	CS3 <i>cs1</i> (1)

Code	†2001 population number	Location	††GPS coordinates	†††mtDNA haplotypes		
				COI	A6-A8	Total-mtDNA
Central Otago continued						
CO.OTA		Transpower Rd, Otamatapaio River Valley	(44°35.00'S, 170°05.20'E)	BW7 (1)	<i>bw1</i> (1)	BW7 <i>bw1</i> (1)
			BW10 (1)	<i>bw2</i> (1)	BW10 <i>bw2</i> (1)	
CO.RSA	[19]	Rastus Burn, The Remarkables Ski Field Road	(45°01.57'S, 168°47.39'E)	CS1 (2)	<i>cs1</i> (2)	CS1 <i>cs1</i> (2)
Otago Lakes						
OL.AIR		Mt Creighton Station 4WD rd, NE edge of Lake Wakatipu	(44°59.91'S, 168°27.17'E)	BSB1 (9)	<i>bsb1</i> (2)	BSB1 <i>bsb1</i> (2)
				CS1 (1)	<i>cs1</i> (1)	CS1 <i>cs1</i> (1)
OL.ALW	[40]	Base of Mt Creighton Station, NE edge of Lake Wakatipu	(45°00.00'S, 168°26.35'E)	BSB1 (1)	<i>bsb1</i> (1)	BSB1 <i>bsb1</i> (1)
				CS1 (1)	<i>cs1</i> (1)	CS1 <i>cs1</i> (1)
OL.BBN		Buckler Burn, N of Lake Wakatipu, just N of Glenorchy	(44°51.57'S, 168°23.57'E)	BSB1 (7)	<i>bsb1</i> (3)	BSB1 <i>bsb1</i> (1)
				BSB2 (1)		BSB2 <i>bsb1</i> (1)
				BSB3 (1)		BSB3 <i>bsb1</i> (1)
OL.CRS		Just off rd, NE edge of Lake Wakatipu, S of Mt Creighton	(45°00.88'S, 168°26.50'E)	BSB1 (2)	<i>bsb1</i> (1)	BSB1 <i>bsb1</i> (1)
OL.DAV	[25]	E side of Dart River, N of L. Wakatipu	(44°46.00'S, 168°20.50'E)	BSB1 (2)	<i>bsb1</i> (1)	BSB1 <i>bsb1</i> (1)
OL.EYR		S side of Eyre Creek, off SH6, near Athol	(45°30.20'S, 168°29.87'E)	CS2 (1)	<i>cs2</i> (2)	CS2 <i>cs2</i> (1)
				CN3 (1)		CN3 <i>cs2</i> (1)
OL.FRL		Mataura river, off SH6, NW of Fairlight	(45°23.85'S, 168°35.61'E)	CS1 (3)	<i>cs1</i> (1)	CS1 <i>cs1</i> (1)
				CS2 (1)	<i>cs2</i> (1);	CS2 <i>cs2</i> (1)
OL.HAB		Makarora River, SH6, S of Haast Pass	(44°09.60'S, 169°17.46'E)	BSB1 (1)	<i>bsa1</i> (2)	BSB1 <i>bsb2</i> (1)
				BSC1 (2)	<i>bsa9</i> (1)	BSC1 <i>bsa1</i> (2)
				BSC3 (1)	<i>bsb2</i> (1)	BSC3 <i>bsa9</i> (1)
OL.INV	[17]	Invincible Mine track, by Rees River, N of Lake Wakatipu	(44°43.87'S, 168°27.36'E)	BSB1 (11)	<i>bsb1</i> (4)	BSB1 <i>bsb1</i> (3)
				BSB4 (1)		BSB4 <i>bsb1</i> (1)
OL.MAR		Mararoa River, at Mararoa River Rd, N of SH94	(45°30.38'S, 167°58.40'E)	BSC2 (5)	<i>bsa1</i> (3)	BSC2 <i>bsa1</i> (3)
				CS1 (1)	<i>cs2</i> (1)	CS1 <i>cs2</i> (1)
OL.NLW	[8]	W side of Lake Hawea, SH6, N of Wanaka	(44°29.00'S, 169°14.85'E)	CN1 (1)	<i>cn2</i> (1)	CN1 <i>cn2</i> (1)
OL.ORE		Oreti river, off SH94, just N of Mossburn	(45°39.75'S, 168°15.08'E)	CS1 (1)	<i>cs2</i> (1)	CS1 <i>cs2</i> (1)
OL.TLL		Near top of Ski Rd up Treble Cone, W of Wanaka	(44°38.14'S, 168°53.72'E)	BSC1 (1)	<i>bsa1</i> (1)	BSC1 <i>bsa1</i> (1)
OL.TWE	[34]	12 Mile Delta, N side Lake Wakatipu, W of Queenstown	(45°04.11'S, 168°32.66'E)	CN1 (7)	<i>cs1</i> (5)	CN1 <i>cs1</i> (1)
				CS1 (1)		CS1 <i>cs1</i> (4)

- † Numbers within square brackets refer to population numbers used by Buckley *et al.* 2001a. The GPS coordinates of these populations may differ from those published in Buckley *et al.* 2001a due to previous miscalculations.
- †† GPS coordinates are given in Lat/Lon as degrees and minutes, using the NZGD49 grid.
- ††† Numbers in parentheses following haplotype refer to number of individuals in that haplotype for that population.

3.2 Molecular labwork: Sequencing and alignment

Divergence levels were relatively low and there were no indels, therefore there was no alignment ambiguity. *M. campbelli* individuals were first sequenced for the COI gene region and aligned to determine their haplotype, then named according to phylogenetic and phylogeographic structure. For each *M. campbelli* with a unique COI sequence and for at least one individual from each population for each COI haplotype, an individual was sequenced for the A6-A8 region and assigned to a unique A6-A8 haplotype (Table 3.1, Appendix 3.4). Additional *M. campbelli* were sequenced in zones identified as potential contact areas between the northern South Island and Otago clades and from preliminary NCA results that indicated areas of particular interest. The following totals include the 35 *M. campbelli* sequenced by Buckley *et al.* (2001a). A total of 753 bp of mtDNA from the 3' end of the COI region was sequenced for 223 *M. campbelli*, and 767 bp of the A6-A8 region, including the whole tRNA^{Asp} (64bp) and A8 (156bp) genes and 554bp of the 3' end of the A6 gene, were sequenced for 154 *M. campbelli*, for a total of 1520bp (Appendix 3.2) for 154 individuals (Appendix 3.4). The non-variable overlapping seven bases at the end of A8 and the start of A6 (found in other insect mtDNA, Buckley *et al.* 2001a) were included with A8 for all analyses.

Haplotype names for COI (upper case) and A6-A8 (lower-case italics) were given an initial letter indicating the main clade to which they belonged (A: North Island, B: northern South Island, C: Otago) and subsequent letters and numbers indicating subclade and individual haplotype. The most common haplotypes were assigned first. Total-mtDNA haplotypes were written as a

combination of COI and A6-A8 haplotype. Abbreviations for clades used throughout the text include: NI (North Island); northern-SI (northern South Island). The northern-SI clades include all South Island clades except Otago, specifically NMK (Nelson, Marlborough and Kaikoura), SA (Southern Alps, with subclades SA-A, SA-B, SA-C (Southern Alps A, etc)) and Waitaki. Appendix 3.2 shows an alignment of all 71 Total-mtDNA haplotypes found. All new sequences will be deposited in Genbank prior to these results being submitted for journal publication.

3.3 Analysis results:

3.3.1 Sequences: base composition and sequence divergences

The Chi square base composition test was negative (15.66215, df=216, $p=1.0000$) indicating that no taxa had significantly different composition of bases compared to the rest. From 1520 bases sequenced there were 115 parsimony informative sites found (Table 3.2). Most of the differences occurred in the third base positions, with only 7.4% of amino acids being variable. The most variable partition (in accordance with other insects (Simon *et al.* 1994)) was the A8 region (Table 3.2).

Appendix 3.3 shows the uncorrected and corrected molecular distances between all Total-mtDNA haplotypes, which are summed up in Table 3.3. Corrected distances were estimated using the ML TrN+I model parameters. The highest sequence divergences are between the Otago haplotypes and all others, as reported by Buckley *et al.* (2001a), with a maximum of 0.045

Table 3.2 *Maoricicada campbelli* mtDNA variation. All values are calculated with the seven overlapping bases at the 3' and 5' end of A8 and A6 respectively included with A8. Gamma values estimated using the method of Frati *et al.* 1997. Maximum-step clades estimated using TCS 1.13.

mtDNA region	No. of individuals sequenced	No. of haplotypes	No. of sites	No. of variable sites (% in brackets)	No. of MP informative sites (% in brackets)	No. of amino acids	No. of variable amino acids (% in brackets)	Gamma value	Maximum-step clades found with NCA
COI 1 st	223	-	251	7 (2.8)	4 (1.6)	-	-	*	-
COI 2 nd	223	-	251	3 (1.2)	0 (0.0)	-	-	*	-
COI 3 rd	223	-	251	60 (23.9)	39 (15.5)	-	-	2.773	-
COI all	223	52	753	70 (9.3)	43 (5.7)	251	9 (3.6)	0.002	NI, NSI, Otago
tRNA ^{Asp}	154	3	64	2 (3.1)	1 (1.6)	-	-	0.014	NI+NSI+Otago
A8 1 st	154	-	52	7 (13.5)	4 (7.7)	-	-	*	-
A8 2 nd	154	-	52	5 (9.6)	4 (7.7)	-	-	*	-
A8 3 rd	154	-	52	7 (13.5)	6 (11.5)	-	-	*	-
A8 all	154	16	156	19 (12.2)	14 (9.0)	52	13 (25.0)	0.647	NI+NSI, Otago
A6 1 st	154	-	182	13 (7.1)	9 (4.9)	-	-	0.019	-
A6 2 nd	154	-	183	4 (2.2)	1 (0.5)	-	-	*	-
A6 3 rd	154	-	182	43 (23.6)	37 (20.3)	-	-	*	-
A6 all	154	31	547	60 (11.0)	47 (8.6)	181	12 (6.6)	0.000	NI, NSI, Otago
Total	154	71	1520	151 (9.9)	105 (6.9)	484	34 (7.0)	0.011	NI, NSI, Otago

* indicates gamma values too large to be estimated.
NI= North Island, NSI = northern South Island.

Table 3.3 Summary of mtDNA distances shown in Appendix 3.3 from 71 Total-mtDNA *Maoricicada campbelli* haplotypes. Uncorrected distances are shown above distances corrected using ML (TrN+I model).

Within and between Total-mtDNA distances for two main clades			Within and between Total-mtDNA distances for three main clades			
	NI+ northern-SI	Otago		NI	northern-SI	Otago
NI+ northern-SI	0.001-0.028 0.001-0.033	0.037-0.045 0.046-0.062	NI	0.001-0.003 0.001-0.003	0.018-0.028 0.020-0.033	0.039-0.043 0.051-0.058
Otago		0.001-0.007 0.001-0.008	northern-SI		0.001-0.018 0.001-0.020	0.037-0.045 0.046-0.062
Within-clade Total-mtDNA distances for subclades						
NMK	SA	Waitaki	Otago north	Otago south		
0.001-0.008 0.001-0.008	0.001-0.007 0.001-0.007	0.001-0.011 0.001-0.011	0.001-0.004 0.001-0.004	0.001-0.004 0.001-0.004		

uncorrected or 0.062 corrected expected number of substitutions per site (genetic distances). This is high for within-species divergence in insects in general, and for other closely related cicadas (Buckley *et al.* 2001a,b). Many other *Maoricicada* species that are well-differentiated in song, habitat and morphology are much less distant in mtDNA (Buckley *et al.* 2001b). The sequence divergence between NI and northern-SI is also relatively high at a maximum of 0.028 uncorrected, 0.033 corrected expected number of substitutions per site. Sequence divergence is very low within all clades except the northern-SI, which has a maximum distance of 0.018 uncorrected, 0.020 corrected expected number of substitutions per site. The northern-SI clade had by far the greatest number of haplotypes and the deepest within-clade structuring.

3.3.2 Phylogenetic results (ML and Bayesian)

A heuristic ML search found one tree, with a -ln likelihood of 3642.5, while the best Bayesian tree found had a -ln likelihood of 3603.2. The Bayesian analysis was found to be stationary with a 10% burn-in (the first 4000 trees), and no autocorrelation was detected between samples. The position of the deep Otago clade as sister to and significantly different from all other *M. campbelli* clades is the strongest phylogenetic result in both the ML and Bayesian analyses (Fig 3.2). This again reinforces the data analysis from Buckley *et al.* (2001a) that indicated that the Otago clade was different enough from all other *M. campbelli* to possibly be a separate species. A small song difference in the Otago *M. campbelli*, discovered while sampling (unpublished data), is being investigated to explore this possibility.

Figure 3.2 Phylograms for 71 *Maoricicada campbelli* Total-mtDNA (COI+A6-A8) haplotypes. **A.** Bayesian topology and branch lengths estimated from a partitioned-model with 4 million generations and a 10% burn-in. Numbers next to nodes represent the Bayesian posterior probabilities. **B.** Maximum likelihood topology and branch lengths estimated from a TrN+I model. Numbers next to nodes represent bootstrap values estimated from 44 pseudoreplicates. Grey highlighting indicates clades found with nested clade analysis, which are not necessarily monophyletic. Names in bold capitals represent the three main clades found in NCA analyses. NI = North Island, SA = Southern Alps, NMK = Nelson, Marlborough and Kaikoura.

The position of the NI clade is ambiguous, and it is possible that this clade is sister to either the NMK clade or the Waitaki+SA clades. Most likely, all three clades split very close in time; neither analysis provides strong support for their respective resolutions (Fig 3.2). The node that separates the NI+Otago and northern-SI clades was only represented in 43% of bootstrap pseudoreplicates (Fig 3.2). This same node was found in 51% of 200 ML and 98% of 500 MP bootstrap pseudoreplicates of a smaller dataset in Buckley *et al.* 2001a. The Bayesian consensus tree differed in only one significant way from the ML tree; in the placement of this NI clade, either as sister to or branching off from within the northern-SI clade (Fig 3.2). The Bayesian analysis showed greater support for the formation of the NI clade after both the NMK and Otago clades had split off (58%) rather than a split with the whole northern-SI clade (42%). These low posterior probabilities indicate significant ambiguity in the data. ML bootstrap support for the most likely Bayesian result with Otago+NMK sister to NI+SA+Waitaki was only 25%.

Most other nodes are reasonably well supported in both ML and Bayesian analyses (Fig 3.2). The five major clades found by Buckley *et al.* (2001a) are all represented in these further analyses, some with additional support from the greater number of haplotypes found. Increased individual/population sampling has resulted in greater ambiguity of the placement of the NI clade, as being either within or sister to the northern-SI clades. Additionally, the ML tree shows only 50% bootstrap support for a monophyletic NMK and the Bayesian tree shows this group as a paraphyletic assemblage that is sister to the remaining non-Otago clades. The increased sampling in the Southern Alps area led to the

SA clade being split into three subclades (SA-A, SA-B, SA-C) (Figs 3.1, 3.2). From the phylogenetic analyses, the SA-B clade is sister to the SA-A+SA-C clades, with Waitaki sister to all SA clades (Fig 3.2). The clades SA-A and SA-B found in the nested clade analyses are not monophyletic in the phylogenetic Bayesian and ML analyses. One haplotype (BSA7bsa11) is sister to all the other SA-A and SA-C haplotypes in both the Bayesian and ML analyses, but is found within the SA-A clade in the nested clade analysis. The haplotype (BSB5bsa7) links the SA-B clade to the SA-A clade in the nested clade analysis (Fig 3.4), but in the phylogenetic analyses this haplotype is part of the main cluster of SA-A haplotypes.

3.3.3 Phylogeographic nested clade analysis results

3.3.3.1 Nested clade analysis network diagrams

Networks were calculated for COI, tRNA^{Asp}, A6 and A8 and the Total mtDNA haplotypes using TCS 1.13. In all NCA haplotype networks except for A8 and tRNA^{Asp}, haplotypes from the NI, northern-SI and Otago clades were separated by a greater number of steps than the 95% parsimony limit (Templeton 1992), which meant NCA could only be performed within each of these major clades (Table 3.2, Figs 3.3, 3.4). For A8, two major clades were found, one including NI+northern-SI, and the other Otago (Table 3.2). For tRNA^{Asp}, all haplotypes were joined together into a single clade (Table 3.2). The small number of individuals sequenced, of variable sites and of haplotypes resulted in the individual NCA analyses of tRNA^{Asp}, A8 and A6 being precluded from further analysis. COI (greater number of individuals) and Total-mtDNA (greater number of haplotypes) NCA networks were analysed further (Figs 3.3, 3.4).

The COI and Total-mtDNA cladograms produced by TCS included ambiguous linkages (Figs 3.3, 3.4). Templeton and Sing (1993) give four possible reasons for recovering ambiguities that reflect data conflict: recombination, scoring errors, incomplete scoring and homoplasy. Mitochondrial DNA, however, has rarely been documented to undergo recombination (Rokas *et al.* 2003) and all DNA sequences were complete and were checked extensively for possible recording errors. The ambiguities recovered from all the TCS cladogram analyses are therefore probably due to homoplasy, where one or more of the mutational sites have undergone further mutations resulting in the loss of data. Additional sequencing of *M. campbelli* would be useful, especially from populations found in geographical areas between clades with currently ambiguous assignments. It is probable that new haplotypes would be found, and some of these may be haplotypes that could make associations between clades clearer.

COI nested clad diagram ambiguities: For the COI cladogram (Fig 3.3), within clade 4-1 (northern South Island) there were two loops leading to ambiguous decisions on associations between clades. The inferred haplotypes in clade 1-Y could have been grouped with clade 2-1, (SA-A), which would have resulted in a single third-level clad grouping everything together. However, coalescent rules state that in cases where ambiguities are caused by odd numbers of clades that the stranded group be grouped with the clad that has the fewest members, thus maximizing the power of the geographical analysis. This meant that clad 1-Y grouped with the Marlborough clad, which in the

Figure 3.3 Nested cladogram of 52 haplotypes of *Maoricicada campbelli* calculated by TCS from 753 bp of the COI mtDNA gene region. The 95% parsimony connection limit = 11 steps. Names represent unique haplotypes, circles represent inferred haplotypes. Straight lines between haplotypes indicate one mutational step. Dashed lines represent alternative connections that were disregarded using information from coalescent theory. Dashed boxes around haplotype names indicate unique tip haplotypes, each one step removed from the interior haplotype. Clade numbers are given as Y-X, with Y= number of the clade level, X= unique number within that level.

next nesting level grouped Kaikoura with the Marlborough clade. Since Kaikoura is more closely related to the Marlborough haplotypes in the phylogenetic analyses, and has fewer mutational steps to the Marlborough clade than any other, this assignment is satisfying. Clade 1-Y could be parsimoniously joined to clade 1-6 through haplotype BW6, and Clade 1-Z could be joined to clade 1-1 through haplotype BSA8. Haplotypes BSA8 and BW6 are each singletons (clades represented by only one individual), which makes them much more likely to be tip clades than internal clades. This means that neither of these associations are viable, and we can dispense with clade 1-Z altogether. To add additional support to this, haplotypes BSA1 and BM1 are both internal clades represented by many individuals, and are much more likely to link the two major clades (3-1 SA+Waitaki and 3-2 NMK) together.

Total-mtDNA nested clade diagram ambiguities: The main ambiguity within the Total-mtDNA diagram (Fig 3.4) is in the connection between the NMK and SA groups. The SA clades could be joined to the rest of the northern-SI clade through either the SA-A or SA-B clades. The phylogenetic trees indicate that SA-B split from Marlborough and Waitaki at the same time as the ancestor to SA-A and SA-C (Fig 3.2). NCA of COI indicates that SA-A is an interior clade (linking to NMK and Waitaki), and SA-B evolved from this (Fig 3.3). However, in the Total-mtDNA diagram, the linking to SA-B through the interior haplotype BSB1*bsb1* makes more sense than linking to SA-A through the inferred haplotype attached to the singleton BSA6*bsa6*. As stated above, it is more likely that an interior clade, rather than a singleton, is linked to other clades. In this case the singleton is not the linking haplotype, but the inferred haplotype

Figure 3.4 Nested cladogram of 71 haplotypes of *Maoricicada campbelli* calculated by TCS from 1520 bp of the COI and A6-A8 mtDNA gene regions. The 95% parsimony connection limit = 17 steps. Names represent unique haplotypes, circles represent inferred haplotypes. Straight lines between haplotypes indicate one mutational step. Dashed lines represent alternative connections that were disregarded using information from coalescent theory. Dashed boxes around haplotype names indicate unique tip haplotypes, each one step removed from the interior haplotype. Clade numbers are given as Y-X, with Y= number of the clade level, X= unique number within that level.

attached to it is. There is still some ambiguity in this decision, which can be cleared up by examining the higher nesting structure. If the NMK and Waitaki haplotypes are joined to the SA clades via the SA-A clade, the SA-A haplotype BSA6*bsa6* remains nested with the inferred haplotypes linking it to the NMK clade. This persists to the fourth nesting level, linking BSA6*bsa6* to part of the Marlborough clade. This is unsatisfactory, because this individual is south of Island Saddle and in the SA-A territory (Fig 3.6). Island Saddle separates all other SA-A and Marlborough haplotypes. In addition to the geographical analysis, BSA6*bsa6* is only two steps removed from the closest SA-A haplotype, yet 13 steps removed from the closest Marlborough haplotype.

The only other ambiguity in the Total-mtDNA diagram is in the linking of haplotypes in the Waitaki clade (Fig 3.4). It is much more likely that BW1*bw1* links through an inferred haplotype to BW1*bw3*, than the singletons BW2*bw1* and BW2*bw3* link the clades together.

3.3.3.2 Nested clade diagram rooting

For the COI cladogram, the haplotypes with the highest outgroup probabilities calculated with TCS were: northern-SI clade (BSA1; outgroup weight 0.19, closest other haplotype (BW1) 0.18; NMK haplotypes 0.00-0.03), Otago clade (CN1; outgroup weight 0.41, closest other haplotype (CS1) 0.41 but fewer connections) and NI clade (A1; outgroup weight 0.88, 0.04 for all other haplotypes).

For the Total-mtDNA cladogram, the haplotypes with the highest outgroup probabilities calculated with TCS were: northern-SI clade (*BSA1bsa1*; outgroup weight 0.14, closest other haplotype (*Bw1bw1*) 0.12; NMK haplotypes 0.00-0.04), Otago clade (*CN1cn1*; outgroup weight 0.36, closest other haplotype (*Cs1cs1*) 0.18) and NI clade (*A1a1*; 0.77, all other haplotypes 0.08). Viewing the geographical ranges finds that in most cases the haplotype with the greatest outgroup weight from the TCS analysis is also the haplotype with the largest geographical range in each clade, which offers further support for the chosen haplotype to be the correct outgroup. In one instance, a different haplotype was found in more populations (*BW1bw1* was found in more populations than *BSA1bsa1*), however this was due to uneven sampling, and the total area covered by the two haplotypes is similar.

The nested cladogram rooting suggested by the coalescent methods differed in every clade to that suggested by the phylogenetic analyses. In the haplotype-rich clades Otago and northern-SI there were several haplotypes (and in the northern-SI clade several subclades) that split off before the haplotype suggested by the coalescent methods as the root. There was not always strong unambiguous support to determine the oldest-branching haplotypes in the phylogenetic trees, however these analyses were able to use information from other clades and outgroups that were too far away to be included in the coalescent methods.

3.3.3.3 Nested clade analysis using geographical associations

Buckley *et al.* (2001a) found that all clades were geographically well defined. The additional sampling from this study supports this, with some clades being separated by only a few km, or sympatric in narrow contact zones (Figs 3.1, 3.6). Additional populations sampled within the published geographical range of any of the clades were always associated with the encompassing clade. New populations extending clade boundaries always extended the range of the predicted clade, with the only uncertain element being in the precise location of zones of contact. The new population from the Nelson area was most closely related to the geographically proximate Marlborough clade, as predicted. The additional populations in southern Otago were from either the Otago clade or the SA-C clade, also as predicted.

The Southern Alps clade was split into three smaller clades, each with a unique geographic range. SA-B, which is sister to SA-A and SA-C, spans the central Southern Alps, while SA-A and SA-C are found at the northern and southern ends of the Southern Alps respectively (Figs 3.1, 3.6). SA-B contacts both other SA clades at their extremes. The Waitaki clade covers much the same area found in the Buckley *et al.* 2001a sampling, however one haplotype belonging to this clade (BW9bw7) was found much farther north (near the Craieburn range, just South of a SA-A population), bridging an unsampled area in the central South Island (Figs 3.1, 3.6).

The historical population movements estimated by the Geodis analyses of COI and Total-mtDNA clades are summarised in Tables 3.4 and 3.5. Appendix 3.5

Figure 3.5 Summary of Geodis output results for NCA of 71 Total-mtDNA *Maoricicada campbelli* haplotypes. Clade names as in Fig 4. As noted elsewhere, the Otago and North Island clades are too divergent to be included in the same nesting sets as the northern South Island clades. See Appendix F for complete raw data. The letter I denotes interior clade, the letter T denotes tip clade. Numbers following clade names are geographical distances for “within clade”, followed by “nested clade”. Letters following geographical distances denote statistical significance, L=large, S=small.

Table 3.4 *Maoricicada campbelli* nested clade geographical associations for COI sequence data estimated using the November 2001 Templeton key and Geodis output data. Clade names as in Fig 3.3.

Nesting level	Clade	Inference key steps	Inference
1	1-2, 1-8, 1-9, 1-11, 1-12, 1-14, 1-15, 1-18	0	No molecular differentiation
	1-5, 1-13	0	No geographical differentiation
	1-1, 1-3, 1-7, 1-10, 1-17, 1-19	1-No	fail to reject H_0 : no geographical structure
	1-4 (part SA-C)	1-2-3-4-9-10-No: <i>M. campbelli</i> probably present between the Homer Tunnel and Boundary Stream.	geographic sampling scheme inadequate to discriminate between fragmentation and isolation by distance
	1-6 (part Waitaki)	1-2-11-12-No	contiguous range expansion
	1-16 (North Island)	1-2-11-17-4-No	restricted gene flow with isolation by distance
	1-20 (part Otago south)	1-2-3-4-No	restricted gene flow with isolation by distance
2	2-2	0	No molecular differentiation
	2-3, 2-6, 2-8	1-No	fail to reject H_0 : no geographical structure
	2-1 (SA-A), 2-4 (Waiaki)	1-2-11-12-No	contiguous range expansion
	2-5 (Marlborough and Nelson)	1-2-3-5-15-16-18-No: <i>M. campbelli</i> possibly present between Cobb Reservoir and Marlborough	geographic sampling scheme inadequate to discriminate between fragmentation, range expansion and isolation by distance (short distance movements).
	2-9 (Otago south)	1-2-3-4-No	restricted gene flow with isolation by distance
3	3-1 (SA and Waitaki)	1-2-11-12-13-Yes	long distance colonisation
	3-2 (NMK)	1-2-11-12-No	contiguous range expansion
	3-4 (Otago)	1-2-No	inconclusive outcome
4	4-1 (northern SI)	1-2-No	inconclusive outcome

Table 3.5 *Maoricicada campbelli* nested clade geographical associations for Total-mtDNA sequence data estimated using the November 2001 Templeton NCA key and Geodis output data. Clade names as in Fig 4. See Fig 5 for a visual representation of clade geographical ranges.

Nesting level	Clade	Inference key steps	Inference
1	1-2, 1-3, 1-6, 1-7, 1-9, 1-11, 1-14, 1-15, 1-16, 1-17, 1-19, 1-21, 1-25, 1-26, 1-27,	0	No molecular differentiation
	1-5, 1-13, 1-24	0	No geographical differentiation
	1-4, 1-8, 1-12, 1-20, 1-28, 1-30, 1-31, 1-34, 1-36	1-No	fail to reject H_0 : no geographical structure
	1-1 (part SA-A and part SA-C)	1-2-3-4-9-10-No: <i>campbelli</i> present but not sampled from the West Coast (Franz Josef, Lake Paringa etc).	geographic sampling scheme inadequate to discriminate between fragmentation and isolation by distance
	1-10 (part Waitaki), 1-18 (part Marlborough)	1-2-11-17-No	inconclusive outcome
	1-22 (part Marlborough)	1-2-11-17-4-9-10-No: <i>campbelli</i> probably present between Rag and Famish Creek and Lake Sedgemere.	geographic sampling scheme inadequate to discriminate between fragmentation and isolation by distance
2	2-2, 2-3, 2-4, 2-6, 2-9, 2-14, 2-15	0	No molecular differentiation
	2-8, 2-13	0	No geographical differentiation
	2-5, 2-11, 2-16, 2-18, 2-19	1-No	Fail to reject H_0 : no geographical structure
	2-1 (part SA-A and SA-C), 2-10 (part Marlborough)	1-2-11-12-No	contiguous range expansion
	2-12 (part Marlborough)	1-2-11-17-No	inconclusive outcome
	2-17 (part Otago)	1-2-11-17-4-9-No	past fragmentation
3	3-5, 3-10	0	No molecular differentiation
	3-1, 3-3, 3-6, 3-8	1-No	fail to reject H_0 : no geographical structure
	3-2 (SA-B)	1-2-11-17-4-No	restricted gene flow with isolation by distance
	3-4 (part Waitaki)	1-2-3-5-6-7-8-No: <i>campbelli</i> may be present between Kye Burn River and Awakino.	geographic sampling scheme inadequate to discriminate between isolation by distance (short distance movements) vs long distance dispersal

shows the Geodis input and raw Geodis output. An output summary for the Total-mtDNA analysis is shown in Fig 3.5. The Geodis geographical associations were visualised by mapping out the current clade ranges (shown here for the Total-mtDNA dataset only, Fig 3.6). Contiguous range expansion was seen in clades where older haplotypes were found throughout the whole range of the clade, while younger haplotypes were found only at the extremities of an apparently expanding edge. Past fragmentation was seen in only one clade that had nested clades with little overlap in their ranges. Restricted gene flow with isolation by distance was seen in clades with some populations a short distance away with some different but reasonably closely related nested clades. Long distance colonization occurred only in two of the largest clades, with the nested clades occupying large non-overlapping territories.

Many of the clades, especially those at the lower-levels, could not be analysed due to the lack of either molecular or geographic structuring (Tables 3.4, 3.5). The clades that did show significant Geodis results were analysed using the 2001 version of the key from Templeton (1995) in conjunction with the maps of the clade locations (Total-mtDNA: Fig 3.6). The new key (Templeton 2004) was also trialled and yielded no different results. Many lower-level and some higher-level clades failed to reject the null hypothesis of no geographical associations (Tables 3.4, 3.5). Several clades were entirely composed of interior or tip clades and had inconclusive outcomes (Tables 3.4, 3.5). Historical population movement of two COI and five Total-mtDNA clades was uncertain because of inadequate sampling (Table 3.5). In each of these cases, *M. campbelli* were probably currently present in an area in between clade ranges that would

otherwise show significant patterning. Such results from earlier analyses on an incomplete dataset were used to determine the most important areas for subsequent field sampling (Templeton 1998, Templeton 2004). The historical population movements for eight COI and seven Total-mtDNA clades were able to be estimated (Tables 3.4, 3.5). The COI dataset had far fewer clades than the Total-mtDNA, however almost 50% more sequenced individuals. This increase in power for the COI dataset is presumably the reason for the reduction in ambiguous clades and the slight increase in positively estimated clades. While following most of the queries in the Templeton (Nov 2001) key led to unambiguous answers, it was at times difficult to interpret the difference between one of the queries that lead to either restricted gene flow with isolation by distance versus past fragmentation (step 4, Templeton Nov 2001 key). While all attempts were made to be impartial, in some cases it was felt that either answer could be correct, as nested clades in most such cases were somewhat overlapping and had medium amounts of genetic difference, and sometimes did not strongly correlate with either scenario.

Total-mtDNA results indicate the northern-SI clade formed through long distance colonisation (Table 3.5), with populations spreading southward from the NMK clade over Island Saddle, a 1365m high drainage-change (Fig 3.6), to populate the Waitaki and Southern Alps areas. Today, the populations in the Marlborough and SA-A clades are completely separated by only ca. 5km over Island Saddle, and yet are only distantly related in mtDNA. The COI data indicates that the NMK clade has undergone contiguous range expansion out from the Marlborough populations to the Nelson and Kaikoura populations

(Table 3.4), and Total-mtDNA data also suggests the southern part of the Marlborough clade has undergone contiguous range expansion (Table 3.5). Most of the SA clades do not have enough data to estimate their geographical history. Some of the clades have significant unsampled areas in between the nested clades that could harbour *M. campbelli*, making it impossible to distinguish between range expansion, fragmentation and isolation by distance (Tables 3.4, 3.5). Total mtDNA indicates that the SA-B clade has undergone restricted gene flow with isolation by distance (Table 3.5), with one individual approximately 150km further north than the rest. This individual is in the same population (NC.ANP, Waimakariri River (Fig 3.1)) as other individuals from the SA-A clade, and in the Total-mtDNA NCA diagram is the haplotype BSB5bsa7 linking SA-B with SA-A (Fig 3.4). Parts of the Waitaki clade have undergone contiguous range expansion from the COI data (Table 3.4). The COI NCA diagram nests Waitaki and the Southern Alps together, and estimates they spread into their current ranges through long distance colonization (Table 3.4).

The North Island clade with the fewest haplotypes and individuals sequenced had mostly non-significant geographical associations. COI data indicated that the most distantly located population (Tukino Ski Field) had undergone restricted gene flow with isolation by distance.

From the Total-mtDNA data, the whole Otago clade had undergone contiguous range expansion, although much of the more northern and central populations underwent range fragmentation at some time. COI data indicated the Otago south clade had undergone restricted gene flow with isolation by distance of the more southern populations.

The Otago clade contacts the northern South Island clades in three different places, and in three different clades, Waitaki, SA-C, and SA-B (Figs 3.1, 3.6). These three contact zones were predicted by Buckley *et al.* (2001a). At Lake Ohau, the northern end of the Otago clade contacts the southwestern edge of the Waitaki clade. The closest populations not in contact are found 12km north (MK.HVI, four individuals sequenced) and 25km south (MK.AHU, 10 individuals sequenced) of this contact zone. At Mt Creighton, on the northcentral edge of Lake Wakitipu, the Otago clade contacts the SA-B clade. The closest populations not in contact are 18km north (OL.BBN, nine individuals sequenced) and 11km south (OL.TWE, eight individuals sequenced) from this contact zone. The single individual found approximately 1.5km south at the OL.CRS collection site was from the SA-C clade, rather than the Otago clade as would have been predicted; OL.CRS is probably a population that is also within this contact zone. These two areas of contact are relatively narrow, measuring only a maximum of approximately 31km (Ohau) and 37km (Mt Creighton). At these two contact zones, haplotypes from both clades were found at both the lowest and highest elevation sampled in the vicinity (Ohau, 538-1500m and Mt Creighton, 400-535m). At Mararora River, south of Queenstown, the southwestern edge of the Otago clade contacts the southeastern edge of the SA-C clade. The closest populations not in contact are found 22km west (FD.BST, one individual sequenced) and 24km east (OL.ORE, one individual sequenced) from this contact zone. The contact zone and nearby populations at Mararora river were the least-well sampled of the three, with this contact zone being a maximum of 46km wide. Further sampling would be necessary to determine the exact width of any of the contact zones,

as in all cases there are many kilometres of unsampled suitable habitat between populations in contact and nearby populations representing a single clade. A fourth contact area predicted by Buckley *et al.* 2001a between the Waitaki and Otago clades in the Omarama area was not found in this study, but would probably be found with further sampling.

Figure 3.6 Total-mtDNA nested clade geographic locations for South Island populations of *Maoricicada campbelli*. The location of all haplotypes and many higher clades are shown. Red with white internal are haplotypes, brown = 1-step clades, blue = 2-step clades, green = 3-step clades, orange = 4-step clades. All localities shown on these two pictures, except for the Otago clades, are joined into the same 5-step clade (5-1, northern-SI clade). Within text boxes white = Southern Alps A and C, blue = Southern Alps B, pink = Waitaki, yellow = NMK and Otago. Some geographical features mentioned in the text are indicated. DEM courtesy of GeographX, NZ.

4. DISCUSSION

4.1 Summary of new *Maoricicada campbelli* findings

The main patterns resolved by the *Maoricicada campbelli* phylogenetic and phylogeographical analyses are 1) the main clades, either two (North Island+northern South Island and Otago), or three (North Island, northern-South Island and Otago), are separated by deep genetic divergence and could represent different species; 2) the population structuring suggests a glacial hypothesis for the distribution of *M. campbelli* throughout the South Island, rather than one mediated by lateral movement along the Alpine Fault; 3) there are three secondary contact zones between the northern South Island and Otago clades, represented by three northern-South Island subclades; and 4) *M. campbelli* in the South Island suggest a conceptual ring species.

4.2 *Maoricicada campbelli* phylogeography and historical population movement

4.2.1 Overall findings

This study employed both phylogenetic (ML and Bayesian) and phylogeographical (NCA) methods of testing relationships within a species. NCA is a method employed to statistically explain the present day range of a species via historical associations of populations, and works best within closely related species with restricted dispersal (Templeton *et al.* 1987). *Maoricicada campbelli* was believed to be a prime candidate for NCA as it was widespread, and initial data (Buckley *et al.* 2001a) indicated a high degree of phylogeographic structuring suggesting a low dispersal rate. These NCA

analyses found three mtDNA divisions within *M. campbelli* (NI, northern-SI and Otago) (Figs 3.3, 3.4) that were too deep to be analysed together. Thus, NCA can shed no light on the historical associations of these main clades, but does provide support for the ML tree where NI is sister to northern-SI, rather than the Bayesian tree that nested NI within the northern-SI clade (Fig 3.2).

Two NCAs were conducted, one on the COI data only, as there were more individuals sequenced for this gene, and one on Total-mtDNA (COI+A6-A8) as there were more haplotypes found which allowed for a finer resolution of geographical associations. Despite the large number of individuals and populations sampled, more data are needed in future to resolve the history of many clades. The COI dataset had almost 50% more individuals sequenced (Table 3.2), and even though it had fewer clades overall, slightly more clades had positive results and fewer clades had ambiguous results (Tables 3.4, 3.5). Sequencing additional *M. campbelli* individuals from populations already sampled in these analyses will increase the power in the geographical associations and in some cases may provide more information for future geographical association analyses than sequencing new populations represented by few individuals. It is possible that some of the geographical associations shown here are false, however most seem intuitive from a visual inspection of clade ranges (Fig 3.6) and a knowledge of New Zealand palaeohistory. NCA estimates should be checked using a second locus, as NCA does not provide estimates of error and is known to fail under certain scenarios (Templeton 2004). The use of multiple loci would guard against incorrect geographical associations, which are unlikely to occur multiple times

(Templeton 2004). Even though there are two NCA analyses shown here, mtDNA is inherited as a unit, therefore different mtDNA genes cannot be used to infer different histories. Sequencing of additional loci to reduce the likelihood of multiple errors in geographical associations is planned for a future study.

4.2.2 Evolution of the North Island clade

The placement of the NI clade in the phylogenetic trees is ambiguous, with low support for the placement of the NI clade either outside or within the NMK clade (Fig 3.2). The NI clade is probably more likely to have dispersed north from a Marlborough clade restricted during glacial periods to populations in northwest Nelson, than to have survived in refuges in the North Island and later spread south to populate the northern South Island. Evidence for a glacial refugium for plants in northwest Nelson is strong (Wardle, 1963) while the southern and central North Island territory that *M. campbelli* could have occupied in the glacial times was less hospitable (McGlone 1988). The fact that *M. campbelli* currently occupies such a restricted range in the North Island, while being found so generally over the South Island supports the hypothesis that *M. campbelli* may not have refuged on the North Island, and may be a recent colonist. However, an alternative explanation could stem from the fact that *M. campbelli* only lives in open habitat, with sparse vegetation, and the North Island is currently more thickly forested than the South Island. *M. campbelli* may have actually been widespread in parts of the North Island during the glacial periods where there were vast open areas of grass and shrubs (McGlone 1988). With the rapid southward movement of forests after the retreat of the glaciers (McGlone 1988), *M. campbelli* could have been subsequently retracted to its

current small range in the open central plateau, similar in method to a retraction in range hypothesised for the alpine scree weta (Trewick *et al.* 2000). One interesting fact, that there are no *Maoricicada* spp. on Mt Taranaki/Egmont may provide evidence that this hypothesis is incorrect, as this relatively open glacial maxima habitat stretched to the Mt Taranaki/Egmont area. The Taranaki volcanoes began to form in the early Pleistocene, with Mt Taranaki the last to form beginning approximately 70,000ya (Thornton 1994). Buckley *et al.* (2001a) dated the NI clade divergence from the northern-SI clade at 0.9my, which would suggest that the NI clade managed to survive in the North Island for some time. Additionally, the NI clade shows relatively deep divergence which cannot be distinguished from a trifurcation involving it, the NMK clade, and the rest of the northern South Island clade (Figs 3.1, 3.2, 3.4), indicating a probable older colonisation event. In the North Island, territory that *M. campbelli* can occupy may overlap the range of the ambiguously related congeneric species *M. iolanthe*, which appears to have a confused history with *M. campbelli* (Buckley *et al.* 2001b). *Maoricicada iolanthe* is unusual in this genus as it does not sing from rocks or short plants on the ground, but rather inhabits trees, which thus restricts its interactions with the ground-dwelling *M. campbelli*. In *M. campbelli* and *M. iolanthe* the songs are almost identical, and if these species did come into contact it is hypothesised that they would attempt to interbreed. A single example of a receptive female *M. campbelli* signalled receptivity to the played-back calls of recorded male *M. iolanthe*, although this female may have been exhibiting increased sexual receptivity seen in older unmated female cicadas (unpublished data). Buckley *et al.* (2001b) found that *M. iolanthe* was positioned within the *M. campbelli* lineages in some

phylogenetic analyses, which could imply mtDNA introgression and indicate hybridisation. Further phylogenetic analyses of all *Maoricicada* species employing multiple genes (in progress) will provide further information on this pattern.

The nested clade analyses involving geographical associations show little significance within the North Island clades due to the lack of individuals sequenced, and from the small clade ranges. The TO.TSR population on the Tukino Ski Field Rd is further (both genetically and geographically) from the other three populations sampled around Mt Ruapehu, and there is indication of restricted gene flow to that population.

4.2.3 Evolution of the northern South Island clades

In the northern-SI clade, populations south of Marlborough were formed by long distance colonisation over Island Saddle. This 1365m junction between peaks constitutes a drainage change and does not harbour *M. campbelli*, even though *M. campbelli* in other locations (for example Mt Ohau) are found at higher altitudes than this. *Maoricicada campbelli* present just to the north and south of the saddle are of very different clades, suggesting a single colonisation event over this barrier. Island Saddle also separates two *Maoricicada mangu* subspecies (Dugdale and Fleming 1978). From the phylogeographical patterning, it appears certain that the southern populations in the northern-SI clade dispersed over Island Saddle prior to the previous interglacial. Dating of these *M. campbelli* clades using molecular clocks should provide further evidence for this hypothesis.

All phylogenetic trees support a split between the Marlborough and the Waitaki clade prior to the splitting off of the SA clade (Fig 3.2). The Waitaki clade has the greatest within-clade genetic distance (Table 3.3), and NCA shows that except for the most northern population (MC.SCR), the northern populations of the Waitaki clade are generally older than the southern ones, indicating a spread southward (Fig 3.6). The most northern population is distantly related to the other Waitaki haplotypes (Fig 3.4), and shows a spread northwards (Fig 3.6). This seems to indicate that the Waitaki population refuged over at least the last glacial maximum south of Island Saddle, possibly in the eastern tundra that was present in the central South Island during the glacial times, a similar habitat to areas on the North Island that are also postulated (above) to have been able to harbour *M. campbelli* throughout glacial periods.

The Southern Alps clades also show evidence of refuging south of northwest Nelson in at least the previous interglacial. NCA indicates that this clade formed though restricted gene flow with isolation by distance from the SA-B subclade to the SA-A+SA-C subclade. The phylogenetic trees show that the SA-B subclade is sister to the SA-A+SA-C subclade (Fig 3.2). Geographically, SA-B is split over the central Southern Alps (Fig 3.6), and NCA estimates that this subclade also formed though restricted gene flow with isolation by distance. Part of the SA-A and SA-C subclades are estimated to have been formed through range expansion. It is possible that the SA-B clade split from Marlborough or early northern Waitaki populations and went south along the Southern Alps during a previous interglacial, and then traveled back north to form the progenitor of the SA-A+SA-C populations. The SA-B clade joins to the

SA-A clade in the Total-mtDNA NCA diagram through a younger haplotype (BSB5bsa7, Fig 3.4) found only in the most northern population (NC.ANP, Fig 3.6). The SA-A clade would then have travelled south to form the closely related SA-C clade. Unsourced populations recorded from Fox and Franz Joseph glaciers may provide evidence for this scenario. The ambiguity seen in the Total-mtDNA diagram where the Marlborough population could be joined to the SA populations through SA-A would indicate a more simple spread southward of the SA-B and SA-C clades from the SA-A clade. Although this scenario is contradicted by the phylogenetic analysis, it can be described as follows: once the SA-A clade was isolated from the Marlborough clade south of Island Saddle, it moved quickly southwards during one of the interglacials down the backbone of the Southern Alps. These populations (SA-B) became isolated from the remaining northern populations (SA-A) during one of the glacial cycles, which did not completely wipe out the SA-B clade refuting in the south. During one of the next interglacials, the SA-A clade again travelled down the backbone of the Southern Alps, and was again wiped out in the central portion to form SA-C clade that also managed to refuge somewhere in the south. Although the likelihood tree has low support for the grouping ((SA-A, SA-C), SA-B), the Bayesian tree shows strong support for this scenario and is preferred because of the increased reality of the partitioned model of evolution (Fig 3.2). The COI NCA cladogram (Fig 3.3) supports linking the NMK clade through the SA-A clade, but has an ambiguity where instead the NMK clade could be joined to the Waitaki clade. Further sequencing of either more individual *M. campbelli* for the same gene regions to attempt to find new linking haplotypes, or a second gene region will be useful to provide stronger support for our preferred hypothesis.

From the NCA, populations in the central Marlborough clade have undergone contiguous range expansion, and COI indicates that the whole NMK clade has undergone contiguous range expansion. This COI NCA result seems unlikely, as the populations in Nelson, Marlborough and Kaikoura are separated by large areas with no sampled *M. campbelli*, and the genetic variation between these clades is high compared to the variation within. Lower-level NMK clades from both COI and Total-mtDNA data find the geographic sampling scheme inadequate to discriminate between fragmentation, range expansion and isolation by distance (short distance movements), because *M. campbelli* may be present in the areas between the Central Marlborough clade and both the Nelson and Kaikoura clades.

4.2.4 Evolution of the Otago clade

The Otago clade has undergone contiguous range expansion, with restricted gene flow to the most southern populations. Even though this clade should have had almost as much time to spread northwards after the retreat of the ice as the northern-SI clade has had to spread southwards, it has spread only a fraction of the distance. If during the last glacial maximum the SA-B and SA-C clades refuged in areas near the southern Southern Alps, and the Waitaki clade refuged in the tundra in the far east of the central South Island, the restricted distribution of the Otago clade could be explained by the short distance between it and the expanding northern-SI clades.

4.3 Nested clade outgroup probabilities and rooting

Rooting nested clade diagrams is considered extremely difficult, and many people do not attempt to root haplotype cladograms (Castelloe and Templeton 1994), instead relying on rooting suggested by phylogenetic methods. The biggest problem is that outgroups usually cannot be considered as they are genetically too distantly related to be included in an intraspecific haplotype network (Crandall and Templeton 1993, Castelloe and Templeton 1994).

Additionally, there is little statistical power to assign outgroup probabilities when dealing with such closely related haplotypes (Castelloe and Templeton 1994).

Coalescent methods, however, can be used to assign outgroup probabilities to haplotypes (Crandall and Templeton 1993, Castelloe and Templeton 1994).

There are several things to consider for each haplotype, including the number of individuals found with that sequence, the position in the cladogram (interior or tip, and distance from midpoint) and the number of other haplotypes it is connected parsimoniously to (Crandall and Templeton 1993). Some problems with using these rooting methods is that uneven sampling can result in a bias towards the interior haplotypes most heavily sampled, which may not always actually be the oldest haplotypes. Using nested clade diagrams in association with phylogenetic methods helps to solve this problem, as phylogenetic trees should be more consistent in the placement of a root (Heulsenbeck *et al.* 2002, but see Castelloe and Templeton 1994).

Outgroup probabilities calculated by TCS consistently placed the interior haplotype with the most connections (also the most frequent haplotype in most cases), as the haplotype with the highest probability of being the root of the

cladogram. This method of cladogram rooting was problematic for all clades as judged by comparison to ML and Bayesian phylogenetic trees. Another way to root nested clade diagrams is to take in to account the number of geographical populations that each haplotype appears in, as an older haplotype should be more generally distributed than a derived one. This geographical rooting method was also found to be unlikely, as it simply corroborated the other coalescent methods. In both the ML and Bayesian phylogenetic trees, the support for exactly which haplotype is sister to the rest of the haplotypes for each main clade is not always strong, however, within the haplotype-rich Otago and northern-SI clades at least, there are many haplotypes that branch off before the one preferred by the coalescent methods as the root. Since the rooting of phylogenetic trees is more believable than that from such coalescent methods, because phylogenetic trees take into account other clades and outgroups, it is therefore far more likely that for each clade a different haplotype that by chance was not well sampled, is the actual root.

4.4 New Zealand biogeography: dispersal versus vicariance

The origins of the flora and fauna of New Zealand is a contentious subject, with on-going debate between proponents of a vicariance method arguing that much of the biota has rafted on New Zealand since the Gondwanan break-up 85mya, and proponents of a dispersal scenario believing that most of the biota has arrived more recently. Most of the origins of the endemic species in the flora and fauna of New Zealand are currently believed to be relatively recent and postdate the Gondwana break-up by many millions of years (e.g., Pole 1994, Winkworth *et al.* 2002). Pole (1994) argued there was evidence that New

Zealand was completely submerged during the Oligocene drowning event approximately 30mya, and therefore that all New Zealand biota must be due to recent dispersal. A few ancient Gondwanan elements such as Kauri (Stöckler *et al.* 2002) and moa (Cooper and Cooper 1995, Cooper *et al.* 2001) are, however, believed to have survived the Oligocene drowning event, corroborating geological evidence that New Zealand has never been fully submerged since its break from Australia (e.g., Cooper and Cooper 1995). The ancestors of all the New Zealand cicadas are thought to have arrived after the Oligocene drowning (Buckley *et al.* 2002, Arensburger *et al.* 2004).

Debate over the origins of distributions within New Zealand also centers on vicariance versus dispersal. Panbiogeographers such as Craw (1988, 1989) and Heads (1998) attempt to explain current distributions of taxa throughout New Zealand by using changes in the landscape over time such as lateral displacement due to the alpine fault. Most other biologists explain current patterns of distribution mostly through dispersal, mediated by the landscape and climatic conditions over time. The most dramatic palaeo-environmental modification in New Zealand has occurred on the South Island, where many distributions and speciation events have been attributed to Southern Alps mountain building since the Pliocene and the Quaternary glacial cycles (e.g., Lockhart *et al.* 2001, McGlone *et al.* 2001, Trewick *et al.* 2000, Trewick and Wallace 2001, Smissen *et al.* 2003, Chin and Gemmel 2004). With the use of molecular techniques, estimates on the age of species and populations are being employed to answer these questions.

4.4.1 Hypotheses on the lack of endemism in the central South Island

Compared to the northern and southern South Island, the central South Island is an endemic-poor area or biotic gap (Cockayne 1926, Willet 1950, Wardle 1963, Craw 1988) (see area marked on Fig 3.1B). There are two main hypotheses to explain this pattern.

4.4.1.1 Glacial hypothesis (dispersal) for the lack of endemism in the central South Island

In the glacial scenario, first proposed by Cockayne (1926) and Willet (1950), glaciers in the Quaternary are hypothesised to have covered much of the narrow central SI, and these combined with mountain building in the same area that produced unstable surfaces and severe glacial outwash resulted in the lack of species seen today. Evidence for severe glaciation in the central Southern Alps during the last (Otira) glaciation ca. 18,000 ya indicates that the snow line was lowered approximately 1000m, and floristic zones lowered approximately 800m (Wardle 1963, Fleming 1980). Previous glacial phases are thought to have been similar (Fleming 1980). Any fauna in severely glaciated areas such as the central Southern Alps would have died out, and the current biota would have recolonised via dispersal from the north or south (Wardle 1963, Fleming 1980, McGlone *et al.* 2001, Trewick and Wallace 2001). This pattern can be seen in data from Trewick and Wallace (2001) who examined invertebrate species with ranges that include this gap area. Phylogenetic analysis of mtDNA suggested that several taxa that were found in the central SI were most closely related to an ancestor in the north or south suggesting that they had recently colonized this area. Additionally, many plant species in this gap area appear to be recent colonists (Wardle 1963, McGlone *et al.* 2001). Some Southern Alps

plant groups have no taxa in the central Southern Alps, e.g. vegetable sheep (*Raoulia* sp., Smissen *et al.* 2003) and alpine Whipcord *Hebe* (Wagstaff & Wardle 1999). Fleming (1980) described two *Maoricicada* species, *M. cassiope* in the north, and *M. clarnitans* in the south that currently almost meet in the central SI, suggesting that these species recently expanded into this area.

4.4.1.2 Alpine Fault hypothesis (vicariance) for the lack of endemism in the central South Island

The Alpine Fault hypothesis as discussed by Craw (1988,1989) and Heads (1998) suggests that lateral movement along the Alpine Fault (see Fig 3.1B) caused species disjunctions and created this gap. Craw (1988: Fig 2) presents an area cladogram, based on the distributions of twelve taxa, that suggests that the northern South Island and southern South Island are most closely related biotically while the central South Island is sister to these. Although it is not stated by Heads or Craw, these sister taxa must be presumed to have refuged somewhere such as the inland tundra or coastal areas of the central South Island during the glacial times. Recent estimates of lateral displacement along the alpine fault have predicted faster movement than previously thought, and there is evidence that the rate of movement has been fastest in the Pliocene/Quaternary (Sutherland 1994). Sutherland (1994) suggests that from the mid-Pliocene (3.4my) there may have been as much as 100km of lateral displacement along the alpine fault. Extrapolating from this and using the fastest predicted rate of lateral movement of 40mm per year (Sutherland 1994) for the whole ca. 200km of central South Island “gap” territory, this predicts that the minimum time for species to become disjunct across this gap would have been around 5my. Taxa found today further to the north or south of this gap

(such as the NMK and Otago *M. campbelli* clades separated by a minimum of ca. 600km) would have taken even longer. From this same rate of movement of 40mm per year it would have taken 12my for the whole 480km of lateral displacement along the alpine fault. It is, however, unlikely that the rate of movement has continuously been this fast (Sutherland 1994).

4.4.1.3 Testing the hypotheses (dispersal versus vicariance) on the lack of endemism in the central South Island

Clearly, depending on the age of the taxa, either scenario (dispersal vs vicariance) is plausible. For related lineages extant in the north and south of the South Island older than 5my (minimum), lateral movement could certainly have contributed to their current distributions. However recent phylogenetic analyses of organisms found on the South Island indicate that many of the lineages showing this disjunct pattern are more closely related than previously thought and postdate most of the lateral slipping (McGlone *et al.* 2001, Trewick and Wallace 2001). For example Wagstaff and Wardle (1999) found 10 species of alpine whipcord *Hebe* that were estimated to have evolved over only ca. 500,000 years. This is a group that grows only on the northern and southern ends of the Southern Alps, even though there is apparently good habitat in the central Southern Alps today (Wagstaff & Wardle 1999). Trewick and Wallace (2001) used a molecular clock to estimate the time of divergence between invertebrate species in 10 genera, finding splitting events across the central South Island gap to be 2-7my. They argued that these estimates postdated the alpine fault hypothesis. Trewick *et al.* (2000), in their study of the South Island alpine weta species *Deinacrida connectens* suggested that even the deep divergence between populations of about 4my was too recent for this

widespread species to have been formed via alpine fault disjunction. Chin and Gemmel (2004) found that reasonably closely related populations of *Celatoblatta* cockroaches were distributed on either side of the Southern Alps, suggesting recent movement after glacial retreat.

Conclusions drawn from phylogeographical patterns of *Maoricicada*

***campbelli* clades overall:** Most of the South Island *M. campbelli* clades were found completely, or almost completely, outside the biotic gap (Fig 3.1). Most populations found inside this gap from clades SA-A, SA-C or Otago were younger and stemmed from recent clade expansions. Almost all the populations inside the gap belonged to the Waitaki and SA-B clades, which spanned the entire biotic gap. The Otago clade and its potentially most close relative the NMK clade were found on either side of the biotic gap, with a minimum distance of ca. 600km between them. Even though they are distantly related (ca. 2.3 ± 0.5 my, Buckley *et al.* 2001a), and may represent separate species, the split between the Otago clade and populations in the northern-SI (and NI) clade is too young to have been influenced much by lateral plate movement along the Alpine Fault. The *M. campbelli* clades that have moved through the biotic gap and come into contact with the Otago clade (Waitaki and SA clades) are descended from the NMK clade or its ancestor. Thus the spread of *M. campbelli* overall fits a glacial hypothesis, with the lineages in the center of the South Island (SA, Waitaki) being more closely related to one of the outer lineages than these outer lineages (NMK, Otago) are to each other.

Conclusions drawn from phylogeographical patterns of *M. campbelli*

clades in the Southern Alps: In addition to the broad history of the *M. campbelli* clades in the South Island, the detailed sampling of *M. campbelli* allows a closer look into the distribution of the populations extant in the Southern Alps. Northern and southern Southern Alps clades (SA-A, SA-C) are more closely related to each other than they are to the central Southern Alps clade (SA-B), especially within the A6-A8 gene where the most common haplotype is found in both SA-A and SA-C. From the phylogenetic trees, Southern Alps B is sister to SA-A+SA-C. This pattern is consistent with the area cladogram shown by Craw (1988: Fig 3.2), however, the estimated time of divergence of less than 1my (Buckley *et al.* 2001a) between these two lineages is far too low for this pattern to be the result of lateral disjunction. The SA-B clade displays a flat comb-like pattern with short branches in the phylogenetic trees, and has only about half the haplotypes of the other two SA clades for the number of individuals sequenced. These factors indicate the SA-B clade has had less time to evolve during modern times and has undergone rapid geographical spread. The locally widespread haplotypes BSA1*bsa1* and BSC1*bsa1* are separated across the central SI gap by only one mutation in the COI gene and by a minimum of ca. 300km. Populations of *M. campbelli* that are yet to be sequenced have been collected from near the Fox and Franz Joseph Glaciers, and should yield further information on the spread of the Southern Alps clades.

Overall conclusions from *Maoricicada campbelli* data on the hypotheses (dispersal vs vicariance) on the lack of endemism in the central South

Island: For all data partitions, *M. campbelli* seems to fit the glacial hypothesis. Even the most ancient divisions within *M. campbelli* appear too young to have been able to be subject to more than 100km of lateral movement, yet are currently a minimum of ca. 600km apart. Thus *M. campbelli* must have been dispersed widely in the late Pliocene, and the populations in the center subsequently wiped out, presumably by glaciers and glacial outwash that were too unstable to support *M. campbelli* populations for long. *Maoricicada campbelli* is a very forgiving species, having a wide altitudinal range from sea level to over 1000m, and is found on a wide range of substrates including dry farm pasture, rocky mountain scree and rocks next to rivers. They can be heard singing in the summer months on the glacial outwash right next to both the Fox and Franz Joseph Glacier rivers. For a species such as this to have been wiped out completely from what was presumably ancient territory indicates that the land must have been very unstable, and climates very unsettled. Because of the relatively deep divergences within the Waitaki clade, there is evidence this clade was able to exist south of Island saddle for at least one glacial cycle and may have refuged in the central South Island during this time. Smissen *et al.* 2003 discuss the possibility that a population of cushion plant (*Raoulia* sp.) was able to refuge in this area over at least the last glacial cycle, and Chin and Gemmel (2004) describe multiple haplotypes in cockroaches found in this central zone that suggest populations may have been able to exist there during glacial maxima. It can also be presumed that every time the glaciers retreated, *M. campbelli* refuging in the north (either on the

North Island or the NMK area, or later in populations further south) and in the Otago area moved towards each other, and populations that came into contact were not able to disperse into each other's territory. Perhaps *M. campbelli* were not able to disperse as far as their current distribution throughout each interglacial, especially during interglacial periods that were cooler and shorter than the current one. Known interglacials as warm as this one have previously occurred (Fleming 1980), and the Waitaki, SA-B and SA-C clades each seem to show a history that must include refuging quite close to the Otago clade. Whether this inability to penetrate into other populations is through an inability to breed, or stems from the fact that the genes disperse too slowly and the peripheral contact populations are always wiped out in the glacial cycles is as yet unknown. There is no evidence of previous contact zones between the Otago and northern-SI clades in the phylogeographical patterning.

4.5 Contact zones

Endler (1977) emphasised that it is not easy to tell whether a contact zone is secondary or primary; a population may split in two and the daughter populations evolve with a stable primary contact zone remaining between them, or a population may be fragmented into two daughter populations by the formation of a barrier, and these spread back around or over the barrier to form a zone of secondary contact. Figure 4.1 details these two possible scenarios, where either 1) populations dispersed to their current clade ranges, or 2) clades evolved in situ via fragmentation. In the fragmentation scenario, contact zones could be primary or secondary, depending whether small amounts of dispersal at clade peripheries was involved, and in the dispersal scenario contact zones would be secondary. Such zones of secondary contact may also stem from

Figure 4.1. Theoretical hypotheses of the distribution of *Maoricadamba campbelli* over ca. 2.3my. Note that the present-day outline of New Zealand is used for all stages without reflecting known landscape changes over this time. Note also that clades are given their present-day range at all times. Ages of Otago and North Island clade separation events estimated by Buckley et al. (2001a). A dispersal scenario predicts that widespread *M. campbelli* were isolated in two refugia during the first glacial cycle, around 2.3mya. Note that the glacial refugium in the NMK area could have been in the North Island. The isolated Otago clade then remained static. The NMK clade dispersed to the North Island (or vice versa) approximately 0.9mya, then the NMK clade dispersed southward into the Waitaki and Southern Alps areas, coming into secondary contact with the Otago clade at the very end of this dispersal. A vicariance scenario predicts that a widespread *M. campbelli* species was subsequently fragmented into isolated populations evolving in situ. The Otago clade was the first to split off ca. 2.3mya, followed by the North Island clade ca. 0.9mya. The NMK clade split off, then lastly the Waitaki and Southern Alps clades separated. In the vicariance hypothesis, which requires the creation of barriers between populations, there would have been small scale dispersal around the edges to create secondary contact zones. Alternatively, population fragmentation could be postulated to follow a parapatric fragmentation model where a strong ecological gradient is coupled with assortative mating related to that gradient. In that case, contact zones would be primary. Inset: *M. campbelli* male.

ring-species where a population does not split, but two expanding edges encircle a barrier and come back into contact (Ridley 1993). Newer phylogenetic and phylogeographic methods (such as NCA), include reconstructions of ancestral areas or geographical data, respectively, to give estimates of types of population movement and we can now predict with more certainty whether we are dealing with primary or secondary contact (Templeton 2001).

Areas where geographically isolated lineages come into secondary contact are common in North America and Europe and are often associated with events surrounding the most recent Quaternary glaciation (Hewitt 1996). Contact zones are less obvious well away from significant geological barriers but have been discovered using genetic techniques (Avice *et al.* 1987). Most contact zones occur between sister species or subspecies, but in several unusual cases more distantly related lineages come into contact, for example, in Herring Gulls (Ridley 1993), in the two 13-year species of the Periodical Cicada *Magicicada* -decim complex (Simon *et al.* 2000, Marshall and Cooley 2000, Cooley *et al.* 2001) and in the now classic ring species of *Ensatina* salamanders (Jackman and Wake 1994, Moritz *et al.* 1992, Wake 1997).

Contact zones are important in studying speciation. Examining mating interactions and searching for hybrid individuals in contact zones can suggest whether populations have evolved sufficient differences to have demonstrable barriers to interbreeding (Ridley 1993). Lineages that meet in contact zones may show reproductive character displacement stimulated by selection associated with postmating barriers to reproduction (Rice and Hostert 1993) and sexual signals that help avoid mistakes in mating (Higgie *et al.* 2000,

Marshall and Cooley 2000, Servedio 2001). Reproductive character displacement occurs when the individuals in contact have evolved strong premating interbreeding barriers but this change has not yet spread into the rest of the population (Waage 1997, Higgie *et al.* 2000, Marshall and Cooley 2000). Alternatively, populations in contact may be interbreeding freely and intermediate forms may be found in the contact areas that are then known as hybrid zones.

In the case of *M. campbelli* we have no answer as yet to whether or not the northern-SI and Otago clades are hybridising at the zones of contact. Unpublished data on song differences between these two clades did not indicate character displacement, and combined with mtDNA evidence may indicate hybridisation. Unpublished data on colour characteristics so far only indicates that individuals seem to be generally more alike within populations than between. The fact that the hybrid zones are narrow, however, does suggest a barrier to gene flow and therefore a lack of (successful) interbreeding between the northern-SI and Otago clades. Arnold (1997) suggested that some hybrid zones are narrow because hybrids are more fit in an intermediate habitat. However, we recognised no difference between the habitat in the contact zones and that just outside them. The narrowness of the contact zones could be due to recency of contact but it seems unlikely that all three contact zones are equally recent.

From the phylogenetic trees (Fig 3.2) we cannot tell whether the *M. campbelli* clades evolved via dispersal or *in situ* from an initial widespread ancestral population (Fig 4.1), which would not allow us to distinguish between primary or

secondary contact at the three contact zones. However, NCA does allow predictions of population history and indicates secondary contact because range expansion is indicated for many clades and long distance dispersal (rather than fragmentation) is indicated for the spreading of populations south of Island Saddle that come onto contact with the Otago clade. The only clade demonstrating past fragmentation from the NCA geographical associations is part of the north-central part of the Otago clade.

4.6 Ring Species

"Ring species" or "circular overlaps", first proposed by Mayr (1963), are originally described from populations that form a more-or-less circular pattern, where populations disperse both ways around a barrier until they meet on the other side, having accumulated sufficient genetic changes along the way to be reproductively isolated. For example, in the North American *Ensatina* salamander complex (Jackman and Wake 1994, Moritz *et al.* 1992, Wake 1997), mountain populations that originated in the north moved south on either side of a central valley experiencing some disjunctions and reconnections over time; the southern-most populations of *Ensatina* that now come into secondary contact are reproductively isolated. Although there is controversy over whether the *Ensatina* clades should be called subspecies or species (Highton 1998), this does not detract from the important evolutionary phenomenon they illustrate. Ring species probably provide the best examples for studies in speciation since they display geographically, instead of over thousands or millions of years, how one species may change into another through isolation by distance (Irwin *et al.* 2001). Ring species demonstrate that small changes over time can lead to the formation of new, reproductively isolated lineages, and that such reproductively

isolated lineages can form even with significant amounts of gene flow around the chain (Irwin *et al.* 2001, Irwin and Irwin 2002). Ring species have been proposed in a number of species complexes, however such classifications are constantly being debated, with alternative views classing them as “meta” or “super” -species or simply groups of species that were not recognised (Martens 1996, Highton 1998, Kvist *et al.* 2003, Liebers 2004). Alternatively, Irwin and Irwin (2002) state that ring species may be more common than currently believed, as some groups that are divided into several species may, in fact, be a single ring species.

The most ideal examples of ring-species are those that are actually physically distributed in a ring, where the contacting reproductively isolated extremes are joined with a circular cline of continuously intergrading populations (Irwin and Irwin 2002). However, a species could be said to be the conceptual equivalent of a ring species if it was formerly widespread and then the central portion was eliminated, isolating the extremes of the range. Later if the central area became habitable again, these extreme populations could expand back into their old locations; if they met in secondary contact zones and were reproductively isolated they would show many of the characteristics of a classic ring species, although some of the linking population chain would have been lost. As most, if not all, examples of ring species are missing some of the intermediate population chain (Irwin and Irwin 2002), conceptual ring species are not as different from the classic circular ring species as one might initially believe. If the linking populations are all missing, we have two unique species. If some of the linking populations are present, then it is possible that the complex may be a ring species.

Maoricicada campbelli is one such example of evolution in a ring-like form, where a widespread ancestor was later isolated into two populations at the extremes of the range when the central area became uninhabitable. These isolated populations then spread inward during the warmer interglacials, and have now met in secondary contact zones and appear to have at least restricted gene flow (Figs 3.1, 3.6, 4.1). A chain of populations in the northern-SI clade spreading from the NMK clade to the lower tips of the SA and Waitaki clades is currently present, as is a small chain of expanded populations within the Otago clade. Although no mating experiments have been conducted, it is probable from the relatively low amount of genetic diversity that successful interbreeding can occur among individuals from all populations within each of the three main clades (NI, northern-SI and Otago). It is unknown, however, whether successful interbreeding could occur between individuals belonging to these clades. In the ring species scenario, the genetically closest populations linking the northern-SI and Otago clades would be from the central Otago area and the central Marlborough (or North Island) area. It is likely, however, that if individuals from different clades within the contact zones are unable to breed, even these somewhat more closely related populations may have barriers to breeding. The genetically closest haplotypes between the Otago clade and the northern-SI clade are separated by 0.038 uncorrected, 0.047 corrected expected number of substitutions per site, high for within-species genetic divergence (Buckley *et al.* 2001a), and much higher than the divergence within any one of the main clades (Table 3.3, Appendix 3.3). If contiguous populations outside the contact zones are able to interbreed, and if the individuals in the contact zones are not, then we will have a good argument for describing *M.*

campbelli as a ring species. Additionally, further analysis of acoustical and morphological data could indicate changes along the chain of linking populations and a ring species-like complex. If populations in the Marlborough area show any evidence of the slight difference in song structure that the Otago clade demonstrates (unpublished data), evidence would be provided for the ring species scenario.

4.7 Potential speciation and song divergence in *Maoricicada campbelli*

Buckley *et al.* (2001a) described many examples of variation in insect mtDNA and concluded that populations of *Maoricicada campbelli* were highly divergent for a single species, with corrected mtDNA divergence between some individuals of over 0.05 expected number of substitutions per site. These analyses confirm this (Table 3.2, Appendix 3.3), and support the hypothesis that the NI, Otago and northern-SI clades, or North Island+northern-SI and Otago could represent cryptic species. Each of these clades inhabits a unique geographical range, except where the northern-SI clade and the Otago clade contact in narrow zones (Figs 3.1, 3.6). Other studies of New Zealand alpine or subalpine taxa have also demonstrated higher-than-usual levels of molecular divergence, sometimes between populations that otherwise show no discernable differences. For example Trewick *et al.* (2000) found that discretely distributed populations of an alpine scree weta, *Deinacrida connectens*, had up to 0.076 uncorrected, 0.130 corrected (GTR+I+Γ) expected number of substitutions per site. Trewick *et al.* (2000) found no other information from nuclear markers that corroborated these findings.

The songs of cicadas facilitate contact between the sexes, and represent the primary isolating mechanism between different species. Cryptic species of cicadas are often discovered first by recognizing a unique call (e.g., Marshall and Cooley 2000, Gogala and Trilar 2004). The *M. campbelli* in the Otago clade have a subtle difference in their song that lends further evidence to the cryptic species hypothesis (unpublished data). However, there is also a suggestion of very small differences between some populations within the northern-SI clades, and so far no song differences have been seen in the North Island (unpublished data). The eastern North American cicada *Magicicada neotredécim* exhibits character displacement in the form of song pitch elevation where it contacts the closely related species *Magicicada tredécim* (Marshall and Cooley 2000). This has been shown to isolate these two species in the contact zone, whereas individuals from outside the contact zone will mate freely (Marshall and Cooley 2000, Simon *et al.* 2000, Cooley *et al.* 2001). No character displacement has been found in preliminary analyses of the songs of *M. campbelli* in the contact zones, however more intensive song analysis will need to be conducted before this is concluded. Individuals from the zones of contact were not known to represent two different clades at the time of collection. Preliminary analyses of individual songs from these populations indicate the song difference may be obscure in such zones, which could indicate hybridization is occurring. Since the *Maoricicada* species exhibit mostly temporal differences in song, rather than pitch differences, it is possible that a more subtle song difference is enough to isolate species, possibly by relying on differences in timed female response. Further work on song structure and mate-choice will be done to determine if the song difference

observed between the northern-SI and Otago clades is isolating or not. One example of a receptive female *M. campbelli* (the same individual that also responded to *M. iolanthe* calls) responded to play-back calls of males of all three main *M. campbelli* clades (unpublished data). The fact that there is only a subtle difference between the songs of these different *M. campbelli* clades (and also that of the ambiguously related *M. iolanthe*) may indicate a difference in cicadas isolated by temporal rather than pitch-mediated song differences. The common ancestor of *Magicicada tredecim* and *M. neotredecim* + *M. septendecim* is estimated to be about a million years old (Simon *et al.* 2000) and *M. tredecim* and *M. neotredecim* show marked character displacement in song when in contact (Marshall and Cooley 2000). The *Maoricicada campbelli* clades presently in contact diverged ca. 2.3mya (Buckley *et al.* 2001a), yet show no evidence of character displacement. Several possible scenarios exist: 1) there is no interbreeding because the small temporal song difference discovered is enough to isolate the clades, 2) the clades are interbreeding freely at the contact zones with no negative effects from hybridisation or 3) the clades are not successfully interbreeding but came into contact too recently for additional song divergence to have occurred. Mate-choice experiments will lend support to one of these hypotheses, and also help to determine the specific status of the main clades. If there was unsuccessful interbreeding between any of the clades, such mating studies could also help break up the trifurcation between the NMK, NI and rest of northern-SI clade, and determine whether two (NI+northern-SI, Otago), three (NI, northern-SI, Otago) or four (NI, NMK, Waitaki+SA, Otago) main clades are present.

5. CONCLUSIONS

5.1 Phylogenetic and phylogeographic population structuring conclusions

The five clades within *Maoricicada campbelli* found by Buckley *et al.* (2001a) were confirmed in this study using both phylogenetic methods and nested clade analysis, with additional resolution into subclades provided by increased population and individual sampling. The placement of these clades into larger clades was problematic, with either two (NI+northern-SI, Otago), three (NI, northern-SI, Otago) or possibly four (NI, NMK, Waitaki+SA, Otago) main clades suggested by different methods of analyses. The population structuring within *M. campbelli* was detailed enough for NCA to be employed, and for estimates of historical movements to be determined for some clades. NCA estimated that the Otago clade has undergone contiguous range expansion, with fragmentation and restricted gene flow to the most southern populations. In the northern-SI clade, populations south of Marlborough were formed by long distance colonisation over the barrier of Island Saddle. Much of the Waitaki, SA-A and NMK clades were formed by range expansion, while some populations within the Waitaki and SA clades were formed through restricted gene flow with isolation by distance.

5.2 Population movements: dispersal versus vicariance conclusions

Almost all *M. campbelli* populations are too recent to have been influenced by vicariance due to Alpine Fault movement, and population structuring of *M. campbelli* within the northern-SI clade is best explained using the glacial theory, that during each Quaternary glacial cycle the center of the South Island is mostly uninhabitable due to heavy glaciation and unstable glacial outwash. The

SA clade is divided into three subclades with mostly non-overlapping ranges, SA-A in the north, SA-B in the center and SA-C in the South, with the phylogenetic relationship (SA-A, SA-C), SA-B. Clades SA-A and SA-C may have occupied their current distributions longer than clade B, which only recently (re)colonised the central Southern Alps. The range of *M. campbelli* during the more recent glaciations, however, was probably not totally restricted to the northwest Nelson and Otago areas as it may have been during the initial glacial maxima. There is evidence from NCA and molecular dating that *M. campbelli* existed on parts of the North Island, eastern-central South Island within the biotic gap, and in two areas near the southern Southern Alps during later glacial maxima.

5.3 Contact zones and ring species conclusions

Three of the four secondary contact zones predicted in Buckley *et al.* (2001a) between the northern-SI and Otago clades were found, and the fourth would likely be found with further sampling. These three contact zones were narrow, and each was represented by a different subclade of the northern-SI clade. The populations in contact represent the younger expanding edge of the northern-SI and Otago clades, populations that are separated by approximately 2.3my (Buckley *et al.* 2001a). As these contact zones represent the young, expanding edges of both clades, *M. campbelli* has a ring-species-like distribution, missing only part of the chain of populations that were wiped out during previous glacial maxima. The narrowness of the contact zones suggests a barrier to geneflow, and that isolation by distance has led to genetic differentiation. Further study is needed to determine whether the different *M.*

campbelli clades are reproductively isolated or hybridising at these areas of contact, and therefore whether they represent different species or one, deeply divergent, species. These studies, as well as additional sampling of *M. campbelli* populations, are currently being undertaken.

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Appendix 2.1 Additional information on molecular labwork methods.

Genomic DNA Extraction: The cicada specimen was removed from the EtOH and placed on a sterile surface. Using sterile forceps, the head and prothorax of the cicada was removed, and then part of the muscle tissue (approx 0.1g) within the mesothorax was pulled out and placed into a 1.5mL microcentrifuge tube containing 0.5mL ddH₂O. After five minutes the ddH₂O was carefully removed with a pipette leaving the rehydrated muscle tissue within the tube.

Three methods of extraction were trialled on two cicada specimens each: 1. the salting out method used by Buckley *et al.* 2001; 2. phenol chloroform; and 3. the Qiagen DNeasy™ Tissue Kit. Timing and safety considerations led to the selection of method 3 for the remainder of the specimens.

PCR amplification: 1. Qiagen Taq DNA Polymerase enzyme and solutions; 2. Applied Biosystems (Perkin Elmer) Gene Amp Amplitaq Gold enzyme and solutions; and 3. Applied Biosystems Amplitaq Gold Master Mix were trialled for the amplification of the ATP gene region. Both methods 1. and 3. were used.

3. For the Amplitaq Gold enzyme and solutions method, specimens were initially amplified on a MJ Research Peltier Thermal Cycler PTC-200 with an annealing temperature gradient across the block from 56-66°C. After viewing the results on a 1% Agarose gel, an annealing temperature of 63°C was used for the rest of the A6-A8 amplifications using this method, and they were run on the same PCR machine. The Amplitaq Gold Master Mix method was run using the two-step method with a ten min initial hold on an Applied Biosystems GeneAmp PCR System 2700.

PCR Amplification regimes:**PCR Amplification of 3' end of the COI gene**

PCR reagents		Cycle Sequencing Protocol	
ddH ₂ O	10.35µL	94°C	2 mins
Q Solution	5.00µL		94°C 45 secs
10x Buffer	2.50µL		56°C 45 secs
dNTP	1.00µL		72°C 1 min 15 secs
Primer 1 (C1-J-2195)	2.00µL		
Primer 2 (TL2-N-3014)	2.00µL		
Qiagen Taq enzyme	0.15µL	72 °C	10 mins
Genomic DNA	2.00µL		
Mineral oil	15µL		

PCR Amplification of the A6-A8 gene region**1) Amplitaq Gold Enzyme and Solutions**

PCR reagents		Cycle Sequencing Protocol	
ddH ₂ O	14.42µL	94°C	2 mins
10x PCR Buffer	2.40µL		94°C 45 secs
MgCl ₂	2.40µL		64°C 45 secs
dNTP	2.00µL		72°C 1 min 15 secs
Primer 1 (TK-J-3799)	1.00µL		
Primer 2 (A6-N-4570)	1.00µL		
AmpliTaq Gold enzyme	0.28µL	72 °C	10 mins
Genomic DNA	1.50µL		

2) Amplitaq Gold Master Mix

PCR reagents		Cycle Sequencing 2-Step Protocol	
ddH ₂ O	7.50µL	95°C	10 mins
Amplitaq Gold Master Mix	12.50µL		95°C 15 secs
Primer 1 (TK-J-3799)	1.50µL		64°C 1 min
Primer 2 (A6-N-4570)	1.50µL		
Genomic DNA	2.00µL	72°C	7 mins

Appendix B. Map of part of insect mitochondrion. Locations of primers and areas sequenced for *Maoricicada campbelli* shown.

Appendix 3.1 Additional information on molecular labwork results.

Genomic DNA Extraction: Two cicadas were extracted using the phenol-chloroform method, two using the salting out method, and 184 using the Qiagen DNEasy tissue Kit. The DNEasy Kit was used as it was quick, safe and the DNA products are believed to have a good storage life. All extractions were visualised on a gel prior to PCR amplification; the first elution was used as the amplification template as there was a greater amount of DNA present.

PCR amplification and purification: The amplification of the COI region, whole and internal, was simple and effective for almost all of the *M. campbelli*. Occasionally an individual had to be re-amplified after viewing the gel, if the band was estimated to be too dim to give good sequencing results. The amplification of A6-A8 was more problematic. Many individuals had to be re-amplified, and the results from two PCRs combined and purified together, to give enough DNA for sequencing. An MJResearch Thermal Cycler was used to optimise the annealing temperature for A6-A8 PCR reactions. Curiously, the result only worked for two of the temperatures attempted, and not for consecutive temperatures. The higher of the two, 63°C was chosen. If the amplification was still problematic, the C3-N-4834 primer used to obtain amplification. This primer was only used towards the end of the molecular labwork, and may actually be a better primer for this region.

PCR products were visualised on a gel prior to purification, and only purified if there was estimated to be enough DNA for sequencing. The final quantity of the elution buffer was estimated from the brightness of the PCR band; for A6-A8 usually only 10µL was used, for COI usually 20µL of elution buffer was used.

Sequencing and alignment: A total of 753 bp of the COI region and 767 bp of the A6-A8 region were aligned and edited. Only sequence clean of ambiguities was used. Because of the length of the amplified region, 20-200bp of sequence at the beginning and end of most alignments was single stranded. If a single stranded end was not a perfectly clean read, the segment was re-sequenced; this was rare, however, if the beginning was unreadable usually the rest of the

sequence was too. Several individuals showed characteristic double peaks throughout their sequence that were presumed to be a large numt. This has been seen in other *Maoricicada* mtDNA sequence (T. Buckley pers. comm.) and in other cicada mtDNA sequence (C. Simon pers. comm.). If the sequence was shown to have double peaks, additional sequence was obtained using the internal primers. The internal primers tended to amplify the mtDNA better than the presumed numt, so the sequence could be determined. If the sequence could not be determined from the internal primers, the individual was not added to the analysis.

Appendix 3.2 Alignment of 71 *Maoricicada campbelli* haplotypes and two outgroups (*Maoricicada clamitans* (M.clamit) and *Maoricicada phaeoptera* (M phaeop) for 753bp of COI and 767bp of A6-A8. Blocks of haplotypes are shaded for ease of reading. The current gene is shown at the top of the page. Codons shaded black are those where the amino acid changes. The current number of bases is shown on the top right corner of each page. Note that codons in A8 are not aligned to spacing.

COI

COI

[illegible]

COI

COI

[illegible]

COI

[180]

[illegible]

COI

COI

[illegible]

COI

COI

[illegible]

COI

[360]

[illegible]

COI

[420]

[illegible]

COI

[illegible]

COI

[illegible]

COI

COI

[illegible]

COI

COI

[illegible]

Appendix D: Halpotype sequences

COI

tRNA^{Asp}

[780]

[illegible]

Appendix D: Halpotype sequences

tRNA^{Asp}

A8

[840]

[illegible]

A8

[900]

[illegible]

A8

[960]

BSA1bsa1	TTT	TGC	TTG	TTT	ATT	AAA	ATC	TAA	TCT	AAT	AAC	TAA	TAA	AAA	GTT	AAT	AGA	TAG	AAT	AAT
BSA1bsa2	...																			
BSA1bsa3	...																			
BSA1bsa4	...																			
BSA1bsa5	...																			
BSA2bsa1	...																			
BSA3bsa1	...																			
BSA4bsa1	...																			
BSA5bsa1	...																			
BSA6bsa6	...						C.													
BSA7bsa11	...								T.											
BSA8bsa1	...																			
BSB1bsb1	...																			
BSB1bsb2	...																			
BSB1bsb3	...																T.			
BSB2bsb1	...																			
BSB3bsb1	...																			
BSB4bsb1	...																			
BSB5bsa7	...																			
BSC1bsa1	...																			
BSC2bsa1	...																			
BSC2bsa8	...																			
BSC3bsa9	...																			
BSC4bsa10	...																			
BSC5bsa1	...																			
BSC6bsa1	...																			
BW1bw1	...							C.	T.											C.
BW1bw3	...							C.	T.											C.
BW1bw5	...							C.												C.
BW2bw1	...							C.	T.											C.
BW2bw3	...							C.	T.											C.
BW3bw2	...							C.	T.											C.
BW4bw1	...							C.	T.											C.
BW5bw4	...							C.												C.
BW6bw1	...							C.	T.											C.
BW7bw1	...							C.	T.											C.
BW8bw6	...							C.												C.
BW9bw7	...							C.												C.
BW10bw2	...							C.	T.											C.
BM1bm1	...							C.												
BM1bm2	...							C.												
BM1bm3	...							C.												
BM1bm4	...							C.												
BM2bm1	...							C.												
BM2bm5	...							C.												
BM3bm1	...							C.												
BM4bm1	...							C.												
BM5bm2	...							C.												
BM6bm6	...							C.												
BN1bn1	...							C.	T.											
BN1bn2	...							C.	T.											
BN2bn1	...							C.	T.											
BN3bn1	...							C.	T.											
BK1bk1	...							C.												
BK2bk2	...							C.												
A1a1	...							C.				G.								
A2a1	...							C.				G.								
A3a1	...							C.				G.								
A4a2	...							C.				G.								
CN1cn1	...		A						T.			G.								G.
CN1cn2	...		A						T.		T	G.								G.
CN1cn3	...		A						T.			G.								G.
CN1cs1	...		A						T.			G.								G.
CN2cn1	...		A						T.			G.								G.
CN3cn2	...		A						T.		T	G.								G.
CN4cn2	...		A						T.		T	G.								G.
CS1cs1	...		A						T.			G.								G.
CS1cs2	...		A						T.			G.								G.
CS2cs2	...		A						T.			G.								G.
CS3cs1	...		A						T.			G.								G.
CS4cs2	...		A						T.			G.								G.
M.clamit	...	A.						C.												
M.phaeop	...	A.						C.												

$[1020]$

	A8		A8/A6		A6										[1020]					
	TTG	AAT	ATG	ATA	ACA	AAT	TTA	TTT	TCA	TCA	TTT	GAT	CCT	TGT	ACT	GGA	TTT	CTT	TCT	CTT
BSA1bsa1																				
BSA1bsa2	...	...	...	...		...						...	...		...	...	...	...	...	...
BSA1bsa3	...					...						...	...		...	...	...	...	...	...
BSA1bsa4	...					...						...	...		...	...	...	...	...	...
BSA1bsa5	...					...						...	...		...	...	...	...	...	...
BSA2bsa1	...					...						...	...		...	...	...	...	...	...
BSA3bsa1	...					...						...	...		...	...	...	...	...	...
BSA4bsa1	...					...						...	...		...	...	...	...	...	...
BSA5bsa1	...					...						...	...		...	...	...	...	...	...
BSA6bsa6	...					...						...	...		...	...	...	...	...	...
BSA7bsa11	...					...						...	...		...	...	...	...	...	...
BSA8bsa1	...					...						...	...		...	...	...	...	...	...
BSB1bsb1	...	...	...	...		...						...	...		...	...	...	...	...	...
BSB1bsb2	...	...	...	...		...						...	...		...	...	...	...	...	...
BSB1bsb3	...					...						...	...		...	...	...	...	...	...
BSB2bsb1	...					...						...	...		...	...	...	...	...	...
BSB3bsb1	...					...						...	...		...	...	...	...	...	...
BSB4bsb1	...					...						...	...		...	...	...	...	...	...
BSB5bsa7	...					...						...	...		...	...	...	...	...	...
BSC1bsa1	...		...	...		...						...	...		...	...	...	...	...	...
BSC2bsa1	...		...	...		...						...	...		...	...	...	...	...	...
BSC2bsa8	...		...	...		...						...	...		...	...	...	...	...	...
BSC3bsa9	...		...	...		...						...	...		...	...	...	...	...	...
BSC4bsa10	...		...	...		...						...	...		...	...	...	...	...	...
BSC5bsa1	...		...	...		...						...	...		...	...	...	...	...	...
BSC6bsa1	...		...	...		...						...	...		...	...	...	...	...	...
BW1bw1	...	...	...	...		...						...	...		...	...	...	...	...	...
BW1bw3	...					...						...	...		...	...	...	...	...	...
BW1bw5	...					...						...	...		...	...	...	...	...	...
BW2bw1	...					...						...	...		...	...	...	...	...	...
BW2bw3	...					...						...	...		...	...	...	...	...	...
BW3bw2	...					...						...	...		...	...	...	...	...	...
BW4bw1	...					...						...	...		...	...	...	...	...	...
BW5bw4	...					...						...	...		...	...	...	...	...	...
BW6bw1	...					...						...	...		...	...	...	...	...	...
BW7bw1	...					...						...	...		...	...	...	...	...	...
BW8bw6	...					...						...	...		...	...	...	...	...	...
BW9bw7	...					...						...	...		...	...	...	T..	...	...
BW10bw2	...					...						...	...		...	...	...	...	...	...
BM1bm1	...		...	...		...						...	...		...	...	...	...	...	...
BM1bm2	...		...	...		...						...	...		...	...	...	...	...	...
BM1bm3	...		...	...		...						...	...		...	...	...	C	...	...
BM1bm4	...		...	...		...						...	...		...	...	...	...	...	...
BM2bm1	...		...	...		...						...	...		...	...	...	...	...	...
BM2bm5	...		...	...		...						...	...		...	...	...	...	...	...
BM3bm1	...		...	...		...						...	...		...	...	...	...	...	...
BM4bm1	...		...	...		...						...	...		...	...	...	...	...	...
BM5bm2	...		...	...		...						...	...		...	...	...	...	...	...
BM6bm6	...		...	...		...						...	...		...	...	...	...	...	...
BN1bn1	...	...	...	...		...						...	...		...	...	...	...	...	...
BN1bn2	...					...						...	...		...	...	...	...	...	...
BN2bn1	...		...	...		...						...	...		...	...	...	...	...	...
BN3bn1	...		...	...		...						...	...		...	...	...	...	...	...
BK1bk1	...		...	...		...						...	...		...	...	...	...	...	...
BK2bk2	...	.C.				...						...	...		...	...	...	...	...	...
A1a1	...		...	...		...						...	...		...	...	...	...	...	...
A2a1	...		...	...		...						...	...		...	...	...	...	...	...
A3a1	...		...	...		...						...	...		...	...	...	...	...	...
A4a2	...		...	...		...						...	...		...	...	...	...	...	...
CN1cn1	...	...	...	...		...						...	...		...	...	...	...	...	...
CN1cn2	...					...						...	...		...	...	...	...	...	...
CN1cn3	...		...	...		...						...	...		...	...	...	...	...	...
CN1cs1	...		...	...		...						...	...		...	...	...	...	...	...
CN2cn1	...		...	...		...						...	...		...	...	...	...	...	...
CN3cn2	...		...	...		...						...	...		...	...	...	...	...	...
CN4cn2	...		...	...		...						...	...		...	...	...	...	...	...
CS1cs1	...		...	...		...						...	...		...	...	...	...	...	...
CS1cs2	...		...	...		...						...	...		...	...	...	...	...	...
CS2cs2	...		...	...		...						...	...		...	...	...	...	...	...
CS3cs1	...		...	...		...						...	...		...	...	...	...	...	...
CS4cs2	...		...	...		...						...	...		...	...	...	...	...	...
M.clamit	...	...	...	...		...						...	...		...	...	...	...	...	...
M.phaeop	...	...	...	...		...						...	...		...	...	...	...	...	...

A6

[1080]

BSA1bsa1	AAT	TGA	TTA	AGA	TCA	ATA	ATT	TTG	TTA	TTA	TTT	TTA	CCT	ATT	ACC	TAT	TGA	TAT	ATG	CCA
BSA1bsa2	...																			
BSA1bsa3	...																			
BSA1bsa4	...																			
BSA1bsa5	...																			
BSA2bsa1	...																			
BSA3bsa1	...																			
BSA4bsa1	...																			
BSA5bsa1	...																			
BSA6bsa6	...																			
BSA7bsa11	...																			
BSA8bsa1	...																			
BSB1bsb1	...																			
BSB1bsb2	...																			
BSB1bsb3	...																			
BSB2bsb1	...																			
BSB3bsb1	...																			
BSB4bsb1	...																			
BSB5bsa7	...																			
BSC1bsa1	...																			
BSC2bsa1	...																			
BSC2bsa8	...																			
BSC3bsa9	...																			
BSC4bsa10	...																			
BSC5bsa1	...																			
BSC6bsa1	...																			
BW1bw1	...													C						
BW1bw3	...													C						
BW1bw5	...													C		T				
BW2bw1	...													C						
BW2bw3	...													C						
BW3bw2	...													C						
BW4bw1	...													C						
BW5bw4	...													C						
BW6bw1	...													C						
BW7bw1	...													C						
BW8bw6	...													C		A				
BW9bw7	...							A						C						
BW10bw2	...													C						
BM1bm1	...													C					A	
BM1bm2	...													C					A	
BM1bm3	...													C					A	
BM1bm4	...													C					A	
BM2bm1	...													C					A	
BM2bm5	...													C					A	
BM3bm1	...													C					A	
BM4bm1	...													C					A	
BM5bm2	...													C					A	
BM6bm6	...													C					A	
BN1bn1	...				.G									C					A	
BN1bn2	...													C					A	
BN2bn1	...				.G									C					A	
BN3bn1	...				.G									C					A	
BK1bk1	...													C					A	
BK2bk2	...													C					A	
A1a1	...										.G			C		T			A	
A2a1	...										.G			C		T			A	
A3a1	...										.G			C		T			A	
A4a2	...										.G			C		T			A	
CN1cn1	...							A			.G		C	.A		T			G	
CN1cn2	...							A			.G		C	.A		T			G	
CN1cn3	...							A			.G		C	.A		T			G	
CN1cs1	...							A			.G		C	.A		T			G	A
CN2cn1	...							A			.G		C	.A		T			G	
CN3cn2	...							A			.G		C	.A		T			G	
CN4cn2	...							A			.G		C	.A		T			G	
CS1cs1	...							A			.G		C	.A		T			G	A
CS1cs2	...							A			.G		C	.A		T			G	
CS2cs2	...							A			.G		C	.A		T			G	
CS3cs1	...							A			.G		C	.A		T			G	A
CS4cs2	...							A			.G		C	.A		T			G	
M.clamit	...	.G						A			.G			T	A		T	.G		A
M.phaeop	...							A			.G			T	A		T			A

A6

[1140]

[illegible]

A6

[1200]

[illegible]

A6

[1260]

[illegible]

A6

[1320]

[illegible]

A6

[1380]

[illegible]

A6

[1440]

[illegible]

A6

[1500]

[illegible]

Appendix D: Halpotype sequences

	A6 [1520]						
BSA1bsa1	AAT	TAT	GAA	TAT	TAT	TCA	GG
BSA1bsa2	...	...	...	...	...	...	..
BSA1bsa3	...	...	...	...	...	.T.	..
BSA1bsa4	...	...	...	...	...	...	..
BSA1bsa5	...	...	...	...	...	...	..
BSA2bsa1	...	...	...	...	...	...	..
BSA3bsa1	...	...	...	...	...	...	..
BSA4bsa1	...	...	...	...	...	...	..
BSA5bsa1	...	...	...	...	...	...	..
BSA6bsa6	...	...	...	...	...	...	..
BSA7bsa11	...	...	...	...	...	...	..
BSA8bsa1	...	...	...	...	...	...	..
BSB1bsb1	...	...	...	...	...	...	..
BSB1bsb2	...	...	...	...	...	...	..
BSB1bsb3	...	...	...	...	...	...	..
BSB2bsb1	...	...	...	...	...	...	..
BSB3bsb1	...	...	...	...	...	...	..
BSB4bsb1	...	...	...	...	...	...	..
BSB5bsa7	...	...	...	...	...	...	..
BSC1bsa1	...	...	...	...	...	...	..
BSC2bsa1	...	...	...	...	...	...	..
BSC2bsa8	...	...	...	...	...	...	..
BSC3bsa9	...	...	...	...	...	...	..
BSC4bsa10	...	...	...	...	...	...	..
BSC5bsa1	...	...	...	...	...	...	..
BSC6bsa1	...	...	...	...	...	...	..
BW1bw1	...	...	.C.	...	...	...	..
BW1bw3	...	...	.C.	...	...	...	..
BW1bw5	...	...	.C.	...	...	...	..
BW2bw1	...	...	.C.	...	...	...	..
BW2bw3	...	...	.C.	...	...	...	..
BW3bw2	...	...	.C.	...	...	...	..
BW4bw1	...	...	.C.	...	...	...	..
BW5bw4	...	...	.C.	...	...	...	..
BW6bw1	...	...	.C.	...	...	...	..
BW7bw1	...	...	.C.	...	...	...	..
BW8bw6	...	...	.C.	...	...	...	..
BW9bw7	...	...	.C.	...	...	...	..
BW10bw2	...	...	.C.	...	...	...	..
BM1bm1	...	...	...	...	...	...	..
BM1bm2	...	...	...	...	...	...	..
BM1bm3	...	...	...	...	...	...	..
BM1bm4	...	...	...	...	...	...	..
BM2bm1	...	...	...	...	...	...	..
BM2bm5	...	...	...	...	...	...	..
BM3bm1	...	...	...	...	...	...	..
BM4bm1	...	...	...	...	...	...	..
BM5bm2	...	...	...	...	...	...	..
BM6bm6	...	...	...	...	...	...	..
BN1bn1	...	...	...	...	...	...	..
BN1bn2	...	...	...	...	...	...	..
BN2bn1	...	...	...	...	...	...	..
BN3bn1	...	...	...	...	...	...	..
BK1bk1	...	...	...	...	...	...	..
BK2bk2	...	...	...	...	...	...	..
A1a1	...	...	...	...	...	...	..
A2a1	...	...	...	...	...	...	..
A3a1	...	...	...	...	...	...	..
A4a2	...	...	...	...	...	...	..
CN1cn1	...	...	...	...	...	...	..
CN1cn2	...	...	...	...	...	...	..
CN1cn3	...	...	...	...	...	...	..
CN1cs1	...	...	...	...	...	...	..
CN2cn1	...	...	...	...	...	...	..
CN3cn2	...	...	...	...	...	...	..
CN4cn2	...	...	...	...	...	...	..
CS1cs1	...	...	...	...	...	...	..
CS1cs2	...	...	...	...	...	...	..
CS2cs2	...	...	...	...	...	...	..
CS3cs1	...	...	...	...	...	...	..
CS4cs2	...	...	...	...	...	...	..
M.clamit	...	...	...	...	...	...	A.
M.phaeop	...	...	...	...	...	...	..

Appendix 3.4 Sequenced individual *Maoricicada campbelli* COI, A6,A8 and Total-mtDNA haplotypes. Refer to Table 1 and Fig 1 for specific locality information.

Clades	Haplotypes			<i>M. campbelli</i> individuals
	COI	A6,A8	TOTAL	
	52 (223)	38 (154)	71 (154)	
NORTH ISLAND (CENTRAL PLATEAU) 4 COI haplotypes 2 A6,A8 haplotypes 4 total-mtDNA haplotypes All haplotypes endemic	A1 (8)			01.TO.BRR.05 01.TO.MAN.03,05 01.TO.BRR.03 01.TO.MAN.01 01.TO.TSR.05 01.TO.TSR.06
		a1 (5)	A1a1 (3)	01.TO.TSR.04
	A2 (1)		A2a1 (1)	98.TO.WSF.15
	A3 (1)		A3a1 (1)	
	A4 (1)	a2 (1)	A4a2 (1)	
MARLBOROUGH 6 COI haplotypes 6 A6,A8 haplotypes 10 total-mtDNA haplotypes All haplotypes endemic	BM1 (4)	bm1 (6*)	BM1bm1 (1)	98.MB.NST.01
		bm2 (2*)	BM1bm2 (1)	98.MB.LSG.36
		bm3 (1)	BM1bm3 (1)	98.MB.KSR.31
		bm4 (1)	BM1bm4 (1)	02.MB.WRR.20
	BM2 (3)	bm1 (6*)	BM2bm1 (2)	01.MB.KOW.02 02.MB.PVS.01
		bm5 (1)	BM2bm5 (1)	98.MB.NST.02
	BM3 (2)	bm1 (6*)	BM3bm1 (2)	98.MB.NST.42 02.MB.WRR.21
	BM4 (1)		BM4bm1 (1)	98.MB.PVS.13
	BM5 (1)	bm2 (2*)	BM5bm2 (1)	98.MB.RFT.04
	BM6 (1)	bm6 (1)	BM6bm6 (1)	02.MB.WRR.22
NELSON 3 COI haplotypes 2 A6,A8 haplotypes 4 total-mtDNA haplotypes All haplotypes endemic	BN1 (3)	bn1 (4*)	BN1bn1 (2)	01.NN.COB.03,05
		bn2 (1)	BN1bn2 (1)	01.NN.COB.01
	BN2 (1)	bn1 (4*)	BN2bn1 (1)	01.NN.COB.02
	BN3 (1)		BN3bn1 (1)	01.NN.COB.04
KAIKOURA 2 COI haplotypes 2 A6,A8 haplotypes 2 total-mtDNA haplotypes All haplotypes endemic	BK1 (1)	bk1 (1)	BK1bk1 (1)	98.KA.ORO.10
	BK2 (1)	bk2 (1)	BK2bk2 (1)	98.KA.WAN.11

Clades	Haplotypes			<i>M. campbelli</i> individuals	
	COI	A6,A8	TOTAL		
SOUTHERN ALPS A Southern Marlborough (Lake Tennyson) to Southern Alps - Arthur's Pass 8 COI haplotypes 7 A6,A8 haplotypes 12 total-mtDNA haplotypes A6,A8 haplotypes <i>bsa</i> unstructured throughout Southern Alps clades 1 A6,A8 haplotype shared with other clades: <i>bsa1</i> – Southern Alps C 1 population with haplotypes also from other clades: NC.ANP – Southern Alps B	BSA1 (21)			98.MB.SRW.01,03 02.MC.PRT.05,06,10	02.NC.ANP.13,14 02.WD.OTR.02
		<i>bsa1</i> (27*)	BSA1 <i>bsa1</i> (8)	01.BR.STJ.03 98.MB.SRW.35 01.MC.CSR.02 02.MC.PRT.03	02.NC.ANP.03 98.NC.EGW.18 98.NC.WGW.16 98.NC.SBH.21
		<i>bsa2</i> (2)	BSA1 <i>bsa2</i> (2)	01.BR.BUR.01,02	
		<i>bsa3</i> (1)	BSA1 <i>bsa3</i> (1)	02.WD.OTR.01	
		<i>bsa4</i> (1)	BSA1 <i>bsa4</i> (1)	98.BR.STJ.14	
		<i>bsa5</i> (1)	BSA1 <i>bsa5</i> (1)	01.BR.BRD.01	
	BSA2 (3)	<i>bsa1</i> (27*)	BSA2 <i>bsa1</i> (3)	01.MC.CSR.03 98.WD.PLC.20	98.WD.ARP.22
	BSA3 (1)		BSA3 <i>bsa1</i> (1)	01.BR.BUR.04	
	BSA4 (1)		BSA4 <i>bsa1</i> (1)	02.NC.ANP.11	
	BSA5 (1)		BSA5 <i>bsa1</i> (1)	01.BR.STJ.02	
	BSA6 (1)	<i>bsa6</i> (1)	BSA6 <i>bsa6</i> (1)	98.MB.LTT.01	
	BSA7 (2)	<i>bsa11</i> (2)	BSA7 <i>bsa11</i> (2)	98.MB.SRW.02	98.MB.LTT.41
	BSA8 (1)	<i>bsa1</i> (27*)	BSA8 <i>bsa1</i> (1)	02.NC.ANP.04	
SOUTHERN ALPS B (Arthurs Pass to Aspiring/North of Lake Wakitipu) 5 COI haplotypes 4 A6,A8 haplotypes 7 total-mtDNA haplotypes All haplotypes endemic; A6,A8 haplotypes <i>bsa</i> unstructured throughout Southern Alps clades 4 populations with haplotypes also from other clades: NC.ANP – Southern Alps A OL.HAB – Southern Alps C OL.AIR – Otago OL.ALW – Otago	BSB1 (33)			02.OL.AIR.02,04,05,06,07,08,09 01.OL.BBN.02,04,06,10,11,12	02.OL.CRS.02 01.OL.INV.02,03,05,11,12,13,14
		<i>bsb1</i> (12*)	BSB1 <i>bsb1</i> (10)	02.OL.AIR.01,03 98.OL.ALW.40 01.OL.BBN.01 02.OL.CRS.01	98.OL.DAV.25 93.OL.INV.17 01.OL.INV.01,04,10
		<i>bsb2</i> (1)	BSB1 <i>bsb2</i> (1)	01.OL.HAB.05	
		<i>bsb3</i> (1)	BSB1 <i>bsb3</i> (1)	02.NC.ANP.02	
	BSB2 (1)	<i>bsb1</i> (12*)	BSB2 <i>bsb1</i> (1)	01.OL.BBN.03	
	BSB3 (1)		BSB3 <i>bsb1</i> (1)	01.OL.BBN.05	
	BSB4 (1)		BSB4 <i>bsb1</i> (1)	01.OL.INV.06	
	BSB5 (1)	<i>bsa7</i> (1)	BSB5 <i>bsa7</i> (1)	02.NC.ANP.12	
SOUTHERN ALPS C (Haast/Fiordland) 6 COI haplotypes 4 A6,A8 haplotypes 7 total-mtDNA haplotypes 1 A6,A8 haplotype shared with other clades: <i>bsa1</i> – Southern Alps A 2 populations with haplotypes also from other clades: OL.HAB – Southern Alps B OL.MAR – Otago	BSC1 (6)	<i>bsa1</i> (27*)	BSC1 <i>bsa1</i> (6)	02.FD.HME.01 01.OL.HAB.01,06	02.OL.TLL.01 01.WD.HAR.01,07
	BSC2 (8)			98.FD.HCT.01	02.OL.MAR.05,06
		<i>bsa1</i> (27*)	BSC2 <i>bsa1</i> (4)	98.FD.BST.43	02.OL.MAR.01,02,03
		<i>bsa8</i> (1)	BSC2 <i>bsa8</i> (1)	98.FD.HCT.30	
	BSC3 (1)	<i>bsa9</i> (1)	BSC3 <i>bsa9</i> (1)	01.OL.HAB.07	
	BSC4 (1)	<i>bsa10</i> (1)	BSC4 <i>bsa10</i> (1)	98.WD.HAR.07	
	BSC5 (1)	<i>bsa1</i> (27*)	BSC5 <i>bsa1</i> (1)	01.WD.HAR.05	
	BSC6 (1)		BSC6 <i>bsa1</i> (1)	01.WD.HAR.06	

Clades	Haplotypes			<i>M. campbelli</i> individuals	
	COI	A6,A8	TOTAL		
WAITAKI 9 COI haplotypes 7 A6,A8 haplotypes 12 total-mtDNA haplotypes All haplotypes endemic 2 populations with haplotypes also from other clades: -MK.LOH – Otago -MK.OHA – Otago	BW1 (37)			01.CO.AWR.02 98.MK.HVI.02,03,04 01.MK.LOH.02,50,51	02.MK.MCV.13 01.MK.RHD.01,03,04,06,10,11,12,13,15
		<i>bw1</i> (22*)	BW1 <i>bw1</i> (17)	98.MK.BCL.33 01.MK.BNO.01 98.MK.HVN.01 98.MK.HVI.01 01.MK.LOH.05,10,12,53,55# 02.MK.LTM.31#	98.MK.MCA.23 98.MK.MCB.27 02.MK.MCR.01 02.MK.MCV.12 02.MK.OHA.23 01.MK.RHD.02,05
		<i>bw3</i> (3*)	BW1 <i>bw3</i> (2)	01.CO.AWR.05,07	
		<i>bw5</i> (1)	BW1 <i>bw5</i> (1)	98.CO.AWR.32	
	BW2 (2)	<i>bw1</i> (22*)	BW2 <i>bw1</i> (1)	01.MK.LOH.06	
		<i>bw3</i> (3*)	BW2 <i>bw3</i> (1)	01.CO.AWR.04	
	BW3 (2)	<i>bw2</i> (3*)	BW3 <i>bw2</i> (2)	01.SC.BWT.01,02	
	BW4 (1)	<i>bw1</i> (22*)	BW4 <i>bw1</i> (1)	01.MK.LOH.56#	
	BW5 (1)	<i>bw4</i> (1)	BW5 <i>bw4</i> (1)	98.CO.KYE.29	
	BW6 (1)	<i>bw1</i> (22*)	BW6 <i>bw1</i> (1)	01.MK.RHD.14	
	BW7 (2)		BW7 <i>bw1</i> (2)	01.CO.AWR.03	98.CO.OTA.01
	BW8 (1)	<i>bw6</i> (1)	BW8 <i>bw6</i> (1)	01.CO.AWR.06	
	BW9 (1)	<i>bw7</i> (1)	BW9 <i>bw7</i> (1)	02.MC.SCR.01	
	BW10(1)	<i>bw2</i> (3*)	BW10 <i>bw2</i> (1)	98.CO.OTA.02	
OTAGO NORTH 4 COI haplotypes 3 A6,A8 haplotypes 7 total-mtDNA haplotypes All haplotypes endemic 1 populations with haplotypes also from another clade: -MK.LOH – Waitaki	CN1(33)			01.CO.CRA.05 01.CO.OMR.02	01.MK.AHU.03,04,10 01.MK.LOH.01,04,08,52,54
		<i>cn1</i> (20*)	CN1 <i>cn1</i> (18)	01.CO.CRA.03 98.CO.LTO.38 01.CO.OMR.01 98.MK.AHU.44 01.MK.AHU.01,02,11,12,13#	98.MK.LIN.28 01.MK.LIN.01,02,10 01.MK.LOH.03,07,09,16 02.MK.OHA.24
		<i>cs1</i> (11*)	Cn1 <i>cs1</i> (1)	01.OL.TWE.04	
		<i>cn2</i> (4*)	CN1 <i>cn2</i> (2)	98.OL.NLW.08	98.CO.ABT.09
		<i>cn3</i> (2)	CN1 <i>cn3</i> (2)	01.MK.LOH.15	98.MK.LOH.37
	CN2 (2)	<i>cn1</i> (20*)	CN2 <i>cn1</i> (2)	01.MK.LOH.11,13	
	CN3 (1)	<i>cn2</i> (4*)	CN3 <i>cn2</i> (1)	01.CO.CRA.09	
	CN4 (1)		CN4 <i>cn2</i> (1)	01.CO.CRA.10	
OTAGO SOUTH 4 COI haplotypes 2 A6,A8 haplotypes 5 total-mtDNA haplotypes All haplotypes endemic 3 populations with haplotypes also from other clades: -OL.AIR – Southern Alps B -OL.ALW – Southern Alps B -OL.MAR – Southern Alps C	CS1 (17)			02.OL.FRL.03,08	OL.TWE.06,07,10
		<i>cs1</i> (11*)	CS1 <i>cs1</i> (10)	01.CO.RSA.01, 98.CO.RSA.19 02.OL.AIR.10 98.OL.ALW.02	02.OL.FRL.01,06 98.OL.TWE.34 01.OL.TWE.01,02,03
		<i>cs2</i> (5*)	CS1 <i>cs2</i> (2)	02.OL.ORE.01	02.OL.MAR.04
	CS2 (2)		CS2 <i>cs2</i> (2)	02.OL.EYR.01	02.OL.FRL.10
	CS3 (1)	<i>cs1</i> (11*)	CS3 <i>cs1</i> (1)	98.CO.OMR.24	
	CS4 (1)	<i>cs2</i> (5*)	CS4 <i>cs2</i> (1)	02.OL.EYR.02	

All numbers in brackets stand for the number of individuals sequenced for that haplotype.

* denotes A6-A8 haplotype elsewhere on table.

All individuals are male unless followed by female symbol (#)

Highlighting denotes individuals sequenced by Buckley *et al.* 2001a. These populations have been renamed with locality codes, the individual number after the code is the same as the original population number published.

Appendix 3.5 Geodis 2.0 input and output for COI and Total-mtDNA analyses of *Maoricicada campbelli* haplotypes. All output based on 10000 resamples.

Population parameters used for all analyses:

number, population code

number of individuals in that population, coordinates (degrees, minutes, decimal seconds)

70 populations

1 TO.BRR	25 MC.PRT	49 CO.ABT
39 10 60 S 175 30 75 E	43 15 81 S 171 42 69 E	44 40 80 S 169 11 50 E
2 TO.MAN	26 MC.SCR	50 CO.AWR
39 08 78 S 175 34 85 E	43 17 75 S 171 43 00 E	44 45 79 S 170 21 62 E
3 TO.TSR	27 SC.BWT	51 CO.CRA
39 17 78 S 175 44 13 E	44 05 91 S 170 33 98 E	44 54 22 S 168 59 10 E
4 TO.WSF	28 MK.AHU	52 CO.KYE
39 13 85 S 175 33 60 E	44 28 79 S 169 43 98 E	45 00 23 S 170 16 45 E
5 MB.KOW	29 MK.BCL	53 CO.LTO
41 42 84 S 173 06 76 E	43 54 86 S 170 33 50 E	44 35 85 S 169 52 80 E
6 MB.KSR	30 MK.BNO	54 CO.OMR
41 36 00 S 173 22 20 E	44 09 81 S 170 06 11 E	45 20 33 S 169 15 26 E
7 MB.LSG	31 MK.HV1	55 CO.OTA
42 08 20 S 172 54 75 E	44 04 80 S 169 49 75 E	44 35 00 S 170 05 20 E
8 MB.LTT	32 MK.HVN	56 CO.RSA
42 12 80 S 172 44 40 E	44 03 30 S 169 49 10 E	45 01 57 S 168 47 39 E
9 MB.NST	33 MK.LIN	57 OL.AIR
41 48 90 S 172 54 85 E	44 35 38 S 169 38 60 E	44 59 91 S 168 27 17 E
10 MB.PVS	34 MK.LOH	58 OL.ALW
41 32 17 S 173 32 59 E	44 14 00 S 169 49 00 E	45 00 00 S 168 26 35 E
11 MB.RFT	35 MK.LTM	59 OL.BBN
42 08 78 S 172 51 08 E	43 39 61 S 170 10 68 E	44 51 57 S 168 23 57 E
12 MB.SRW	36 MK.MCA	60 OL.CRS
42 11 14 S 172 46 82 E	44 06 00 S 170 08 00 E	45 00 88 S 168 26 50 E
13 MB.WRR	37 MK.MCB	61 OL.DAV
41 43 21 S 173 05 75 E	43 44 30 S 170 04 45 E	44 46 00 S 168 20 50 E
14 NN.COB	38 MK.MCR	62 OL.EYR
41 06 35 S 172 41 20 E	43 48 68 S 170 06 72 E	45 30 20 S 168 29 87 E
15 BR.BRD	39 MK.MCV	63 OL.FRL
42 22 00 S 172 21 00 E	43 44 11 S 170 05 73 E	45 23 85 S 168 35 61 E
16 BR.BUR	40 MK.OHA	64 OL.HAB
42 04 93 S 172 14 24 E	44 13 70 S 169 46 85 E	44 09 60 S 169 17 46 E
17 BR.STJ	41 MK.RHD	65 OL.INV
42 21 85 S 172 24 00 E	44 11 83 S 170 05 79 E	44 43 87 S 168 27 36 E
18 KA.ORO	42 WD.ARP	66 OL.MAR
42 30 20 S 173 29 15 E	42 54 60 S 171 33 60 E	45 30 38 S 167 58 40 E
19 KA.WAN	43 WD.HAR	67 OL.NLW
42 30 56 S 173 08 42 E	44 05 90 S 169 21 76 E	44 29 00 S 169 14 85 E
20 NC.ANP	44 WD.OTR	68 OL.ORE
43 01 58 S 171 37 47 E	42 51 20 S 171 33 62 E	45 39 75 S 168 15 08 E
21 NC.EGW	45 WD.PLC	69 OL.TLL
42 34 90 S 172 35 20 E	42 53 79 S 171 33 56 E	44 38 14 S 168 53 72 E
22 NC.SBH	46 FD.BST	70 OL.TWE
42 46 35 S 172 10 90 E	45 18 30 S 167 47 75 E	45 04 11 S 168 32 66 E
23 NC.WGW	47 FD.HCT	
42 34 60 S 172 27 75 E	45 14 40 S 167 48 85 E	
24 MC.CSR	48 FD.HME	
43 09 16 S 171 44 25 E	44 45 97 S 167 59 35 E	

***M. campbelli* COI Geodis 2.0 input matrices:**

21

1-1 SOUTHERN ALPS A (1)

7

BSA1 BSA2 BSA3 BSA4 BSA5 BSA6 BSA8

0 1 1 1 1 1 1

14

8 12 15 16 17 20 21 22 23 24 25 42 44 45

0 3 1 2 2 3 1 1 1 1 4 0 2 0

0 0 0 0 0 0 0 0 0 1 0 1 0 1

0 0 0 1 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 1 0 0 0 0 0 0 0 0

0 0 0 0 1 0 0 0 0 0 0 0 0 0

1 0 0 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 1 0 0 0 0 0 0 0 0

1-3 SOUTHERN ALPS B (2)

5

BSB1 BSB2 BSB3 BSB4 BSB5

0 1 1 1 1

8

20 57 58 59 60 61 64 65

1 9 1 7 2 1 1 11

0 0 0 1 0 0 0 0

0 0 0 1 0 0 0 0

0 0 0 0 0 0 0 1

1 0 0 0 0 0 0 0

1-4 SOUTHERN ALPS C (3)

4

BSC1 BSC2 BSC3 BSC4

0 1 1 1

7

43 46 47 48 64 66 69

2 0 0 1 2 0 1

0 1 2 0 0 5 0

0 0 0 0 1 0 0

1 0 0 0 0 0 0

1-6 WAITAKI #1 (4)

6

BW1 BW2 BW4 BW5 BW6 BW7

0 1 1 1 1 1

15

29 30 31 32 34 35 36 37 38 39 40 41 50 52 55

1 1 4 1 8 1 1 1 1 2 1 11 4 0 0

0 0 0 0 1 0 0 0 0 0 0 0 1 0 0

0 0 0 0 1 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0 0 1 0

0 0 0 0 0 0 0 0 0 0 0 1 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0 1 0 1

1-7 WAITAKI #2 (5)

2

BW3 BW10

0 1

2

27 55

2 0

0 1

1-10 MARLBOROUGH (6)

4

BM1 BM2 BM3 BM5

0 0 1 1

7

5 6 7 9 10 11 13

0 1 1 1 0 0 1

1 0 0 1 1 0 0
0 0 0 1 0 0 1
0 0 0 0 0 1 0

1-16 NORTH ISLAND (7)

4
A1 A2 A3 A4
0 1 1 1

4
1 2 3 4
2 3 3 0
0 0 0 1
0 0 1 0
0 0 1 0

1-17 NORTH OTAGO (8)

3
CN1 CN2 CN3
0 1 1

10
28 33 34 40 49 51 53 54 67 70
9 4 11 1 1 2 1 2 1 1
0 0 2 0 0 0 0 0 0 0
0 0 0 0 0 1 0 0 0 0

1-19 SOUTH OTAGO #1 (9)

2
CS1 CS3
0 1

6
54 56 57 58 63 70
0 2 1 1 4 7
1 0 0 0 0 0

1-20 SOUTH OTAGO #2 (10)

2
CS2 CS4
0 1

2
62 63
1 1
1 0

2-1 SOUTHERN ALPS A (11)

2
1-1 1-2
0 1
14
8 12 15 16 17 20 21 22 23 24 25 42 44 45
1 3 1 3 3 5 1 1 1 1 4 1 2 1
1 1 0 0 0 0 0 0 0 0 0 0 0 0

2-3 SOUTHERN ALPS C (12)

2
1-4 1-5
0 1

7
43 46 47 48 64 66 69
3 1 2 1 3 5 1
2 0 0 0 0 0 0

2-4 WAITAKI (13)

4
1-6 1-7 1-8 1-9
0 1 1 1
17
26 27 29 30 31 32 34 35 36 37 38 39 40 41 50 52 55
0 0 1 1 4 1 10 1 1 1 1 2 1 12 6 1 1
0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

2-5 MARLBOROUGH + NELSON (14)

4
1-10 1-11 1-12 1-13
0 1 1 1

8
5 6 7 9 10 11 13 14
1 1 1 3 1 1 2 0
0 0 0 0 1 0 0 0
0 0 0 0 0 0 1 0
0 0 0 0 0 0 0 5

2-6 KAIKOURA (15)

2
1-14 1-15
1 0

2
18 19
1 0
0 1

2-8 NORTH OTAGO (16)

2
1-17 1-18
0 1
10
28 33 34 40 49 51 53 54 67 70
9 4 13 1 1 3 1 2 1 1
0 0 0 0 0 1 0 0 0 0

2-9 SOUTH OTAGO (17)

2
1-19 1-20
0 1
7
54 56 57 58 62 63 70
1 2 1 1 0 4 7
0 0 0 0 2 1 0

3-1 SOUTHERN ALPS + WAITAKI (18)

4
2-1 2-2 2-3 2-4
0 1 1 1
44
8 12 15 16 17 20 21 22 23 24 25 26 27 29 30 31 32 34 35 36 37 38 39 40 41 42 43 44
2 4 1 3 3 5 1 1 1 1 4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 2
0 0 0 0 0 2 0
0 5 0
0 0 0 0 0 0 0 0 0 0 0 1 2 1 1 4 1 10 1 1 1 1 2 1 12 0 0 0 0
45 46 47 48 50 52 55 57 58 59 60 61 64 65 66 69
1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 9 1 9 2 1 1 12 0 0
0 1 2 1 3 5 1 0 0 0 0 0 3 0 5 1
0 0 0 0 7 1 2 0 0 0 0 0 0 0 0 0

3-2 NORTHERN SOUTH ISLAND (19)

2
2-5 2-6
0 1
10
5 6 7 9 10 11 13 14 18 19
1 1 1 3 2 1 3 5 0 0
0 0 0 0 0 0 0 0 1 1

3-4 OTAGO (20)

2
2-8 2-9
0 0
15
28 33 34 40 49 51 53 54 56 57 58 62 63 67 70
9 4 13 1 1 4 1 2 0 0 0 0 0 1 1
0 0 0 0 0 0 0 1 2 1 1 2 1 0 7

4-1 SOUTH ISLAND (NOT OTAGO) (21)

```

2
3-1 3-2
0 0
53
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 29 30 31 32 34 35
0 0 0 2 0 0 0 4 0 0 1 3 3 0 0 5 1 1 1 1 4 1 2 1 1 4 1 10 1
1 1 1 0 3 2 1 0 3 5 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0
36 37 38 39 40 41 42 43 44 45 46 47 48 50 52 55 57 58 59 60 61 64 66 69
1 1 1 2 1 12 1 5 2 1 1 2 1 10 6 3 9 1 9 2 1 4 5 1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
END

```

***M. campbelli* Geodis COI analysis output results:**

PERMUTATION ANALYSIS OF 1-1 SOUTHERN ALPS B (1)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 79.9111

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.4892

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BSA1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	46.5923	0.0800	0.9200
NESTED CLADE	48.2494	0.1897	0.8103

CLADE BSA2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	9.7724	0.0598	0.9402
NESTED CLADE	51.1969	0.4122	0.5878

CLADE BSA3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8651	1.0000
NESTED CLADE	67.0174	0.7213	0.3811

CLADE BSA4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8635	1.0000
NESTED CLADE	53.5540	0.5465	0.4535

CLADE BSA5 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8652	1.0000
NESTED CLADE	43.1348	0.2390	0.8612

CLADE BSA6 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8711	1.0000
NESTED CLADE	74.5877	0.8645	0.1355

CLADE BSA8 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8597	1.0000
NESTED CLADE	53.5540	0.5555	0.4445

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	42.9276	0.8183	0.1817
NESTED CLADE	-7.4305	0.3960	0.6040

PERMUTATION ANALYSIS OF 1-3 SOUTHERN ALPS B (2)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 26.4731

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3967

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BSB1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	29.1220	0.1333	0.8667
NESTED CLADE	33.4517	0.1544	0.8456

CLADE BSB2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.7575	1.0000
NESTED CLADE	19.8770	0.5650	0.6775

CLADE BSB3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.7582	1.0000
NESTED CLADE	19.8770	0.5705	0.6713

CLADE BSB4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.7656	1.0000
NESTED CLADE	12.3973	0.3312	1.0000

CLADE BSB5 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.7545	1.0000
NESTED CLADE	309.1238	1.0000	0.0527

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	29.1220	0.1483	0.8517
NESTED CLADE	-56.8671	0.1747	0.8253

PERMUTATION ANALYSIS OF 1-4 SOUTHERN APLS C (3)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 23.1111

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3141

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BSC1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	48.5242	0.1451	0.8549
NESTED CLADE	62.6613	0.3081	0.6919

CLADE BSC2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	12.2653	0.0000	1.0000
NESTED CLADE	66.7235	0.4447	0.5553

CLADE BSC3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	107.5348	0.6190	0.3810

CLADE BSC4 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	0.0000	1.0000	1.0000	
NESTED CLADE	116.3522	0.8116	0.1884	

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	38.7120	0.9109	0.0891	
NESTED CLADE	-13.1063	0.2855	0.7145	

PERMUTATION ANALYSIS OF 1-6 WAITAKI #1 (4)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 79.0216

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3884

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BW1 (Interior)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	29.2423	0.0121	0.9879	
NESTED CLADE	30.3618	0.0247	0.9753	

CLADE BW2 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	26.4809	0.7814	0.2186	
NESTED CLADE	63.1864	0.9418	0.0582	

CLADE BW4 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	0.0000	0.6580	1.0000	
NESTED CLADE	26.3939	0.6594	0.5610	

CLADE BW5 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	0.0000	0.6588	1.0000	
NESTED CLADE	98.5282	0.9774	0.0226	

CLADE BW6 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	0.0000	0.6635	1.0000	
NESTED CLADE	8.7998	0.0474	0.9526	

CLADE BW7 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	10.5012	0.6395	0.3669	
NESTED CLADE	55.9542	0.8731	0.1269	

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	18.6760	0.0561	0.9439	
NESTED CLADE	-22.7815	0.0224	0.9776	

PERMUTATION ANALYSIS OF 1-7 WAITAKI #2 (5)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 3.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3291

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BW3 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.3291	1.0000
NESTED CLADE	21.8437	0.3291	1.0000

CLADE BW10 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	43.6253	0.6709	0.3291

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.3291	1.0000
NESTED CLADE	-21.7816	0.3291	0.6709

PERMUTATION ANALYSIS OF 1-10 MARLBOROUGH (6)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 19.0278

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.5887

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BM1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	30.3075	0.7720	0.2280
NESTED CLADE	30.1989	0.6305	0.3695

CLADE BM2 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	18.2770	0.4245	0.5755
NESTED CLADE	23.7911	0.2922	0.7078

CLADE BM3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	9.3997	0.2467	0.7533
NESTED CLADE	13.4961	0.1989	0.8011

CLADE BM5 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.6963	1.0000
NESTED CLADE	39.7849	0.8976	0.1024

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	18.8851	0.7594	0.2406
NESTED CLADE	5.1937	0.6508	0.3492

PERMUTATION ANALYSIS OF 1-16 NORTH ISLAND (7)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 13.7500

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.2488

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE A1 (Interior)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	8.4522	0.5151	0.4849	
NESTED CLADE	9.4412	0.7270	0.2730	

CLADE A2 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	0.0000	1.0000	1.0000	
NESTED CLADE	3.8441	0.0000	1.0000	

CLADE A3 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	0.0000	1.0000	1.0000	
NESTED CLADE	14.7872	0.5417	0.4583	

CLADE A4 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	0.0000	1.0000	1.0000	
NESTED CLADE	14.7872	0.5481	0.4519	

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	8.4522	0.5151	0.4849	
NESTED CLADE	-1.6983	0.6378	0.3622	

PERMUTATION ANALYSIS OF 1-17 NORTH OTAGO (8)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 14.9650

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.4970

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE CN1 (Interior)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	38.6993	0.2608	0.7392	
NESTED CLADE	38.8070	0.2624	0.7376	

CLADE CN2 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	0.0000	0.1979	1.0000	
NESTED CLADE	56.3321	0.7347	0.2653	

CLADE CN3 (Tip)				
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	0.0000	0.9731	1.0000	
NESTED CLADE	45.1219	0.5294	0.4706	

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=	
WITHIN CLADE	38.6993	0.8052	0.1948	
NESTED CLADE	-13.7884	0.2385	0.7615	

PERMUTATION ANALYSIS OF 1-19 SOUTH OTAGO #1 (9)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 16.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.1816

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE CS1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	18.7031	0.0000	1.0000
NESTED CLADE	20.7631	0.0000	1.0000

CLADE CS3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.5605	1.0000
NESTED CLADE	48.0342	1.0000	0.0592

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	18.7031	0.0000	1.0000
NESTED CLADE	-27.2711	0.0000	1.0000

PERMUTATION ANALYSIS OF 1-20 SOUTH OTAGO #2 (10)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 0.7500

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE CS2 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	5.3569	1.0000	0.0000
NESTED CLADE	4.6874	1.0000	0.6614

CLADE CS4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	2.1871	0.6614	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	5.3569	1.0000	0.0000
NESTED CLADE	2.5003	1.0000	0.6614

PERMUTATION ANALYSIS OF 2-1 SOUTHERN ALPS A (11)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 9.9107

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.6293

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	49.8765	0.1603	0.8397
NESTED CLADE	49.7641	0.0409	0.9591

CLADE 1-2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	2.3863	0.0971	0.9217
NESTED CLADE	69.9223	0.8733	0.1455

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	47.4901	0.9029	0.0971
NESTED CLADE	-20.1582	0.1455	0.8545

PERMUTATION ANALYSIS OF 2-3 SOUTHERN ALPS C (12)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 5.8500

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.5151

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-4 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	68.0736	0.1175	0.8825
NESTED CLADE	69.1575	0.0673	0.9327

CLADE 1-5 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.1617	1.0000
NESTED CLADE	109.3031	0.9327	0.0673

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	68.0736	0.8569	0.1431
NESTED CLADE	-40.1456	0.0673	0.9327

PERMUTATION ANALYSIS OF 2-4 WAITAKI (13)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 95.3220

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0504

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-6 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	37.0400	0.0093	0.9907
NESTED CLADE	39.9541	0.0409	0.9591

CLADE 1-7 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	29.1043	0.6810	0.3190
NESTED CLADE	36.2873	0.4277	0.5723

CLADE 1-8 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.6986	1.0000
NESTED CLADE	75.8706	0.9625	0.1849

CLADE 1-9 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.6980	1.0000

NESTED CLADE	147.2767	1.0000	0.0184
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PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	19.5774	0.0966	0.9034
NESTED CLADE	-26.4478	0.0709	0.9291

PERMUTATION ANALYSIS OF 2-5 MARLBOROUGH + NELSON (14)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 29.1833

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.1868

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-10 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	28.0427	0.1254	0.8746
NESTED CLADE	27.9774	0.1116	0.8884

CLADE 1-11 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8251	1.0000
NESTED CLADE	45.2513	0.5889	0.5297

CLADE 1-12 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8305	1.0000
NESTED CLADE	3.1475	0.1860	0.8140

CLADE 1-13 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.0001	1.0000
NESTED CLADE	75.4033	1.0000	0.0001

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	28.0427	0.9561	0.0439
NESTED CLADE	-32.7962	0.0027	0.9973

PERMUTATION ANALYSIS OF 2-6 KAIKOURA (15)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 2.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-14 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	14.0352	0.5024	0.4976

CLADE 1-15 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	14.0341	0.4976	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	-0.0011	0.4976	0.5024

PERMUTATION ANALYSIS OF 2-8 NORTH OTAGO (16)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 8.4792

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3991

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-17 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	39.2240	0.1099	0.8901
NESTED CLADE	39.4145	0.1099	0.8901

CLADE 1-18 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.9722	1.0000
NESTED CLADE	43.6531	0.5376	0.5705

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	39.2240	0.1099	0.8901
NESTED CLADE	-4.2386	0.4624	0.5376

PERMUTATION ANALYSIS OF 2-9 SOUTH OTAGO (17)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 12.9833

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0447

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-19 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	24.3752	0.0695	0.9305
NESTED CLADE	26.8019	0.1886	0.8114

CLADE 1-20 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	3.6456	0.0601	0.9465
NESTED CLADE	30.0101	0.8175	0.1825

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	20.7296	0.8951	0.1049
NESTED CLADE	-3.2082	0.1825	0.8175

PERMUTATION ANALYSIS OF 3-1 SOUTHERN ALPS + WAITAKI (18)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 380.2256

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN

OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	50.9082	0.0000	1.0000
NESTED CLADE	210.4752	1.0000	0.0000

CLADE 2-2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	39.1682	0.0000	1.0000
NESTED CLADE	175.3378	0.9940	0.0060

CLADE 2-3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	88.4700	0.0183	0.9817
NESTED CLADE	144.9838	0.6955	0.3045

CLADE 2-4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	46.6660	0.0000	1.0000
NESTED CLADE	48.9781	0.0000	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-3.2913	0.4512	0.5488
NESTED CLADE	97.1833	1.0000	0.0000

PERMUTATION ANALYSIS OF 3-2 NMK (19)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 19.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0659

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-5 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	33.9507	0.0065	0.9935
NESTED CLADE	38.2908	0.0000	1.0000

CLADE 2-6 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	14.0347	0.2669	0.7396
NESTED CLADE	72.9158	0.8807	0.1193

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	19.9160	0.5667	0.4333
NESTED CLADE	-34.6250	0.0840	0.9160

PERMUTATION ANALYSIS OF 3-4 OTAGO (20)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 44.4889

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-8 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	39.5523	0.0000	1.0000
NESTED CLADE	48.2555	0.0703	0.9297

CLADE 2-9 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	28.4126	0.0023	0.9977
NESTED CLADE	59.1841	0.8264	0.1736

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

PERMUTATION ANALYSIS OF 4-1 SOUTH ISLAND (NOT OTAGO) (21)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 148.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 3-1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	134.4319	0.0000	1.0000
NESTED CLADE	150.6586	0.0000	1.0000

CLADE 3-2 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	45.2158	0.0000	1.0000
NESTED CLADE	269.0434	1.0000	0.0000

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

***M. campbelli* Geodis Total-mtDNA input:**

26

1-1 SOUTHERN ALPS A&C1 (1)

11

BSA1bsa1	BSA1bsa2	BSA1bsa3	BSA1bsa4	BSA1bsa5	BSA2bsa1	BSA3bsa1	BSA4bsa1	BSA5bsa1
0	1	1	1	1	1	1	1	1
BSA8bsa1	BSC1bsa1							
1	0							

17

12	15	16	17	20	21	22	23	24	25	42	43	44	45	48	64	69
1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	2	1

1-4 SOUTHERN ALPS C 1

2

BSC2bsa1	BSC2bsa8
0	1

3

46 47 66

1	0	3
0	1	0

2-1 SOUTHERN ALPS A&C2 (2)

5

1-1	1-3	1-4	1-5	1-6	1-7
0	1	1	1	1	1

21

8	12	15	16	17	20	21	22	23	24	25	42	43	44	45	46	47	48	64	66	69
0	2	1	3	2	3	1	1	1	2	1	1	2	1	1	0	0	1	2	0	1
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	3	0
0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

3-1 SOUTHERN ALPS a&c3 (3)

2

2-1 2-2

0	1
---	---

21

8	12	15	16	17	20	21	22	23	24	25	42	43	44	45	46	47	48	64	66	69
1	2	1	5	2	3	1	1	1	2	1	1	5	1	1	1	1	1	3	3	1
1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1-8 SOUTHERN ALPS B1 (4)

6

BSB1bsb1	BSB1bsb2	BSB1bsb3	BSB2bsb1	BSB3bsb1	BSB4bsb1
0	1	1	1	1	1

8

20	57	58	59	60	61	64	65
0	2	1	1	1	1	0	4
0	0	0	0	0	0	1	0
1	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0
0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	1

3-2 SOUTHERN ALPS B2 (5)

2

2-3 2-4

1 0
 8
 20 57 58 59 60 61 64 65
 1 2 1 3 1 1 1 5
 1 0 0 0 0 0 0 0
4-1 SOUTHERN ALPS ALL (6)
 2
 3-1 3-3
 0 1
 27
 8 12 15 16 17 20 21 22 23 24 25 42 43 44 45 46 47 48 57 58 59 60 61 64 65 66 69
 2 3 1 5 2 3 1 1 1 2 1 1 5 1 1 1 1 1 0 0 0 0 0 3 0 3 1
 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 2 1 3 1 1 1 5 0 0
1-10 WAITAKI 1 (7)
 4
 BW1bw1 BW2bw1 BW4bw1 BW7bw1
 0 1 1 1
 14
 29 30 31 32 34 35 36 37 38 39 40 41 50 55
 1 1 1 1 5 1 1 1 1 1 1 2 0 0
 0 0 0 0 1 0 0 0 0 0 0 0 0 0
 0 0 0 0 1 0 0 0 0 0 0 0 0 0
 0 0 0 0 0 0 0 0 0 0 0 0 1 1
1-12 WAITAKI 2 (8)
 2
 BW3bw2 BW10bw2
 0 1
 2
 27 55
 2 0
 0 1
2-5 WAITAKI 3 (9)
 2
 1-10 1-11
 0 0
 14
 29 30 31 32 34 35 36 37 38 39 40 41 50 55
 1 1 1 1 7 1 1 1 1 1 1 2 1 1
 0 0 0 0 0 0 0 0 0 0 0 1 0 0
2-7 WAITAKI 4 (10)
 2
 1-13 1-14
 0 0
 2
 50 52
 3 0
 0 1
3-3 WAITAKI 5 (11)
 2
 2-5 2-6
 0 1
 15
 27 29 30 31 32 34 35 36 37 38 39 40 41 50 55
 0 1 1 1 1 7 1 1 1 1 1 1 3 1 1
 2 0 0 0 0 0 0 0 0 0 0 0 0 0 1
3-4 WAITAKI 6 (12)
 2
 2-7 2-8
 0 1
 2
 50 52
 3 1
 2 0
4-2 WAITAKI ALL (13)
 3

3-3 3-4 3-5
 0 1 1
 17
 26 27 29 30 31 32 34 35 36 37 38 39 40 41 50 52 55
 0 2 1 1 1 1 7 1 1 1 1 1 1 3 1 0 1
 0 0 0 0 0 0 0 0 0 0 0 0 0 0 5 1 0
 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1-18 MARLBOROUGH 1 (14)
 3
 BM1bm1 BM1bm4 BM3bm1
 0 1 1
 2
 9 13
 1 0
 0 1
 1 1
1-20 MARLBOROUGH 3 (15)
 2
 BM2bm1 BM2bm5
 0 1
 3
 5 9 10
 1 0 1
 0 1 0
1-22 MARLBOROUGH 2 (16)
 2
 BM1bm2 BM5bm2
 0 1
 2
 7 11
 1 0
 0 1
2-10 MARLBOROUGH 4 (17)
 2
 1-18 1-19
 0 1
 3
 6 9 13
 0 2 2
 1 0 0
2-11 MARLBOROUGH 5 (18)
 2
 1-20 1-21
 0 1
 3
 5 9 10
 1 1 1
 0 0 1
2-12 MARLBOROUGH 6 (19)
 2
 1-22 1-23
 0 1
 3
 7 11 13
 1 1 0
 0 0 1
3-6 MARLBOROUGH 7 (20)
 2
 2-10 2-11
 0 1
 5
 5 6 9 10 13
 0 1 2 0 2
 1 0 1 2 0
3-7 MARLBOROUGH 8 (21)

2
 2-12 2-13
 0 1
 4
 7 11 13 14
 1 1 1 0
 0 0 0 5
3-8 KAIKOURA (22)
 2
 2-14 2-15
 1 0
 2
 18 19
 1 0
 0 1
4-4 MARLBOROUGH 7 + NELSON + KAIKOURA (23)
 2
 3-7 3-8
 0 1
 6
 7 11 13 14 18 19
 1 1 1 5 1 0
 0 0 0 0 0 1
5-1 ALL SOUTH ISLAND (24)
 4
 4-1 4-2 4-3 4-4
 1 1 0 1
 53
 5 6 7 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 29 30 31 32 34 35
 0 0 0 0 0 0 3 0 0 1 5 2 0 0 5 1 1 1 2 1 0 0 0 0 0 0 0
 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 2 1 1 1 1 7 1
 1 1 0 3 2 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
 0 0 1 0 0 1 0 1 5 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0
 36 37 38 39 40 41 42 43 44 45 46 47 48 50 52 55 57 58 59 60 61 64 65 66 69
 0 0 0 0 0 0 1 5 1 1 1 1 1 0 0 0 2 1 3 1 1 4 5 3 1
 1 1 1 1 1 3 0 0 0 0 0 0 0 6 1 1 0 0 0 0 0 0 0 0
 0
 0
1-28 NORTH ISLAND 1
 3
 A1a1 A2a1 A3a1
 0 1 1
 4
 1 2 3 4
 1 1 1 0
 0 0 0 1
 0 0 1 0
2-16 NORTH ISLAND ALL
 2
 1-28 1-29
 1 1
 4
 1 2 3 4
 1 1 2 1
 0 0 1 0
1-30 OTAGO NORTH 1
 3
 CN1cn1 CN1cn3 CN2cn1
 0 1 1
 6
 28 33 34 40 53 54
 6 4 4 1 1 1
 0 0 2 0 0 0
 0 0 2 0 0 0
1-31 OTAGO NORTH 2

```

2
CN1cn2 CN4cn2
  0      1
3
49 51 67
  1  0  1
  0  1  0
1-34 OTAGO SOUTH 1
2
CS1cs1 CS3cs1
  0      1
6
54 56 57 58 63 70
  0  2  1  1  2  4
  1  0  0  0  0  0
1-36 OTAGO SOUTH 2
2
CS2cs2 CS4cs2
  0      1
2
62 63
  1  1
  1  0
2-17 OTAGO 3
3
1-30 1-33 1-34
  0    0    0
11
28 33 34 40 53 54 56 57 58 63 70
  6  4  8  1  1  1  0  0  0  0  0
  0  0  0  0  0  0  0  0  0  0  1
  0  0  0  0  0  1  2  1  1  2  4
2-18 OTAGO 4
2
1-31 1-32
  0    1
3
49 51 67
  1  1  1
  0  1  0
2-19 OTAGO 5
2
1-35 1-36
  0    1
4
62 63 66 68
  0  0  1  1
  2  1  0  0
3-10 OTAGO ALL
3
2-17 2-18 2-19
  0    1    1
17
28 33 34 40 49 51 53 54 56 57 58 62 63 66 67 68 70
  6  4  8  1  0  0  1  2  2  1  1  0  2  0  0  0  5
  0  0  0  0  1  2  0  0  0  0  0  0  0  0  1  0  0
  0  0  0  0  0  0  0  0  0  0  0  2  1  1  0  1  0
END

```

***M. campbelli* Geodis Total-mtDNA output results:**

PERMUTATION ANALYSIS OF 1-1 SOUTHERN ALPS A&C1 (1)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 161.9583

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.6018

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BSA1bsa1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	33.7826	0.0048	0.9952
NESTED CLADE	89.8720	0.2466	0.7534

CLADE BSA1bsa2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.0301	1.0000
NESTED CLADE	131.2241	0.6781	0.3310

CLADE BSA1bsa3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8840	1.0000
NESTED CLADE	30.1246	0.1946	0.8054

CLADE BSA1bsa4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8863	1.0000
NESTED CLADE	153.4694	0.6866	0.3134

CLADE BSA1bsa5 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8820	1.0000
NESTED CLADE	113.3430	0.4254	0.5746

CLADE BSA2bsa1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	9.7724	0.0193	0.9807
NESTED CLADE	26.7616	0.0065	0.9935

CLADE BSA3bsa1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8850	1.0000
NESTED CLADE	131.2241	0.6935	0.4172

CLADE BSA4bsa1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8806	1.0000
NESTED CLADE	23.0271	0.1144	1.0000

CLADE BSA5bsa1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8806	1.0000
NESTED CLADE	115.7834	0.5061	0.4939

CLADE BSA8bsa1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8862	1.0000
NESTED CLADE	23.0271	0.1142	1.0000

CLADE BSC1bsa1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	48.5242	0.1598	0.8402

NESTED CLADE	265.9588	1.0000	0.0000
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PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	37.6573	0.1693	0.8307
NESTED CLADE	87.6102	0.9859	0.0141

PERMUTATION ANALYSIS OF 1-4 SOUTHERN ALPS C 1

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 5.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.4035

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BSC2bsa1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	11.6585	0.5965	0.4035
NESTED CLADE	10.5987	0.7986	0.4035

CLADE BSC2bsa8 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	11.1687	0.4035	0.7986

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	11.6585	0.5965	0.4035
NESTED CLADE	-0.5700	0.7986	0.4035

PERMUTATION ANALYSIS OF 2-1 SOUTHERN ALPS A&C2 (2)
BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 100.4703

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.5207

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	119.5629	0.0332	0.9668
NESTED CLADE	143.3585	0.0223	0.9777

CLADE 1-3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	192.7435	0.7641	0.2658

CLADE 1-4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	188.9250	0.7999	0.2001
NESTED CLADE	292.0379	1.0000	0.0000

CLADE 1-5 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.0457	1.0000
NESTED CLADE	151.7744	0.4059	0.6088

CLADE 1-6 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	160.3769	0.6040	0.4741

CLADE 1-7 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	151.7744	0.4199	0.7086

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	9.3567	0.1029	0.8971
NESTED CLADE	-94.3672	0.0027	0.9973

PERMUTATION ANALYSIS OF 3-1 SOUTHERN ALPS A&C (3)
BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 15.4386

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.5903

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	168.2964	0.5541	0.4539
NESTED CLADE	165.7079	0.2653	0.7427

CLADE 2-2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	2.3863	0.0598	0.9482
NESTED CLADE	186.4657	0.7328	0.2752

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	165.9100	0.9558	0.0522
NESTED CLADE	-20.7578	0.2762	0.7318

PERMUTATION ANALYSIS OF 1-8 SOUTHERN ALPS B1 (4)

PART I. EXACT CONTINGENCY TEST: OBSERVED CHI-SQUARE STATISTIC = 40.8000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3874

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BSB1bsb1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	13.9633	0.0923	0.9077
NESTED CLADE	25.0298	0.0690	0.9310

CLADE BSB1bsb2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8006	1.0000
NESTED CLADE	83.8699	0.9319	0.0681

CLADE BSB1bsb3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8027	1.0000
NESTED CLADE	307.2901	1.0000	0.0675

CLADE BSB2bsb1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.7935	1.0000
NESTED CLADE	21.6570	0.5984	0.6081

CLADE BSB3bsb1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.7893	1.0000
NESTED CLADE	21.6570	0.6025	0.6082

CLADE BSB4bsb1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.7995	1.0000
NESTED CLADE	11.9714	0.3338	0.6662

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	13.9633	0.0976	0.9024
NESTED CLADE	-64.2593	0.0713	0.9287

PERMUTATION ANALYSIS OF 3-2 SOUTHERN ALPS B2 (5)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 7.4667

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.5037

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-3 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	40.7353	0.1224	0.8776
NESTED CLADE	49.3336	0.0000	1.0000

CLADE 2-4 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8099	1.0000
NESTED CLADE	294.9406	1.0000	0.1224

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
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WITHIN CLADE	-40.7353	0.8776	0.1224
NESTED CLADE	245.6070	1.0000	0.0000

PERMUTATION ANALYSIS OF 4-1 SOUTHERN ALPS ALL (6)
 BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 45.5469

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
 OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 3-1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	167.7887	0.0710	0.9290
NESTED CLADE	180.9400	0.2285	0.7715

CLADE 3-2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	59.2913	0.0000	1.0000
NESTED CLADE	210.1687	0.9369	0.0631

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	108.4975	0.9957	0.0043
NESTED CLADE	-29.2287	0.0999	0.9001

PERMUTATION ANALYSIS OF 1-10 WAITAKI 1 (7)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 25.2353

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
 OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.9075

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BW1bw1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	26.1783	0.0699	0.9301
NESTED CLADE	26.4452	0.0491	0.9509

CLADE BW2bw1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8149	1.0000
NESTED CLADE	34.5905	0.7601	0.5798

CLADE BW4bw1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8103	1.0000
NESTED CLADE	34.5905	0.7574	0.5583

CLADE BW7bw1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
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WITHIN CLADE	10.5012	0.6993	0.3038
NESTED CLADE	70.0491	0.9665	0.0335

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	20.9277	0.2389	0.7611
NESTED CLADE	-25.8746	0.0239	0.9761

PERMUTATION ANALYSIS OF 1-12 WAITAKI 2 (8)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 3.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3373

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BW3bw2 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.3373	1.0000
NESTED CLADE	21.8437	0.3373	1.0000

CLADE BW10bw2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	43.6253	0.6627	0.3373

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.3373	1.0000
NESTED CLADE	-21.7816	0.3373	0.6627

PERMUTATION ANALYSIS OF 2-5 WAITAKI 3 (BOTH INTERIOR?) (9)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 6.6349

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.6807

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-10 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	29.5144	0.6375	0.3625
NESTED CLADE	29.5134	0.5937	0.4063

CLADE 1-11 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.7720	1.0000
NESTED CLADE	22.7487	0.2728	0.8639

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

PERMUTATION ANALYSIS OF 2-7 WAITAKI 4(10)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 4.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.2460

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-13 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.2460	1.0000
NESTED CLADE	18.8662	1.0000	0.2460

CLADE 1-14 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	8.0844	0.2460	1.0000

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

PERMUTATION ANALYSIS OF 3-3 WAITAKI 5 (11)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 20.2652

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.1072

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-5 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	29.4537	0.0551	0.9449
NESTED CLADE	29.8331	0.0583	0.9417

CLADE 2-6 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	29.1043	0.7945	0.2055
NESTED CLADE	43.7783	0.8340	0.1660

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.3494	0.1764	0.8236
NESTED CLADE	-13.9453	0.1660	0.8340

PERMUTATION ANALYSIS OF 3-4 WAITAKI 6 (12)

BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 0.6000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-7 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	14.8257	1.0000	0.0000
NESTED CLADE	16.4730	0.6705	1.0000

CLADE 2-8 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.6705	1.0000
NESTED CLADE	20.5927	1.0000	0.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	14.8257	1.0000	0.0000
NESTED CLADE	-4.1196	0.0000	1.0000

PERMUTATION ANALYSIS OF 4-2 WAITAKI ALL (13)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 56.6181

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0102

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 3-3 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	30.3831	0.0009	0.9991
NESTED CLADE	33.2401	0.0007	0.9993

CLADE 3-4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	13.1009	0.0110	0.9890
NESTED CLADE	92.7303	0.9666	0.0334

CLADE 3-5 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.8364	1.0000
NESTED CLADE	143.0661	1.0000	0.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	19.1538	0.4653	0.5347
NESTED CLADE	-66.6810	0.0037	0.9963

PERMUTATION ANALYSIS OF 1-18 MARLBOROUGH 1 (14)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 2.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BM1bm1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.4889	1.0000
NESTED CLADE	9.3974	0.0000	1.0000

CLADE BM1bm4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.5008	1.0000
NESTED CLADE	9.4021	1.0000	0.5008

CLADE BM3bm1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	9.3997	0.3349	0.6651
NESTED CLADE	9.3997	0.1623	0.8377

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-6.2665	0.3266	0.6734
NESTED CLADE	-0.0032	0.0000	1.0000

PERMUTATION ANALYSIS OF 1-20 MARLBOROUGH 3 (15)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 3.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.6687

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BM2bm1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	18.2688	0.6622	0.6687
NESTED CLADE	16.2397	0.6622	0.6687

CLADE BM2bm5 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.6691	1.0000
NESTED CLADE	27.4448	0.3378	0.6622

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	18.2688	0.6622	0.3378
NESTED CLADE	-11.2051	0.6622	0.3378

PERMUTATION ANALYSIS OF 1-22 MARLBOROUGH 2 (16)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 2.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE BM1bm2 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	2.9641	1.0000	0.4944

CLADE BM5bm2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	2.9637	0.0000	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	0.0003	1.0000	0.0000

PERMUTATION ANALYSIS OF 2-10 MARLBOROUGH 4 (17)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 5.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.1922

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-18 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	9.3997	0.0000	1.0000
NESTED CLADE	15.2055	0.1922	0.8078

CLADE 1-19 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.6010	1.0000
NESTED CLADE	20.2620	0.4088	0.5912

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	9.3997	0.0000	1.0000
NESTED CLADE	-5.0566	0.5912	0.4088

PERMUTATION ANALYSIS OF 2-11 MARLBOROUGH 5 (18)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 1.3333

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-20 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	18.2770	0.0000	1.0000
NESTED CLADE	21.5365	0.7506	0.2494

CLADE 1-21 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.7495	1.0000

NESTED CLADE	26.3391	0.7495	0.2505
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PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	18.2770	0.0000	1.0000
NESTED CLADE	-4.8027	0.2505	0.7495

PERMUTATION ANALYSIS OF 2-12 MARLBOROUGH 6 (19)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 3.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-22 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	2.9639	0.0000	1.0000
NESTED CLADE	7.5943	0.3306	1.0000

CLADE 1-23 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	43.2585	1.0000	0.3306

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	2.9639	0.0000	1.0000
NESTED CLADE	-35.6641	0.3306	1.0000

PERMUTATION ANALYSIS OF 3-6 MARLBOROUGH 7 (20)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 6.3000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.2865

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-10 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	17.3726	0.4904	0.5096
NESTED CLADE	18.1013	0.2918	0.7082

CLADE 2-11 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	22.5656	0.8247	0.2014
NESTED CLADE	21.8459	0.6463	0.3537

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-5.1930	0.2840	0.7160
NESTED CLADE	-3.7447	0.3061	0.6939

PERMUTATION ANALYSIS OF 3-7 MARLBOROUGH 8 (21)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 8.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0143

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-12 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	12.6892	0.1971	0.8172
NESTED CLADE	37.0896	0.0143	0.9857

CLADE 2-13 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.0143	1.0000
NESTED CLADE	77.1033	1.0000	0.0143

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	12.6892	1.0000	0.0143
NESTED CLADE	-40.0137	0.0143	0.9857

PERMUTATION ANALYSIS OF 3-8 KAIKOURA (22)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 2.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-14 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	14.0352	0.4902	0.5098

CLADE 2-15 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	14.0341	0.5098	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	-0.0011	0.5098	0.4902

PERMUTATION ANALYSIS OF 4-4 MARLBOROUGH 7 + NELSON + KAIKOURA (23)

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 10.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.2987

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 3-7 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	52.1232	0.7011	0.2989
NESTED CLADE	49.3869	0.6989	0.3011

CLADE 3-8 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	51.3219	0.3011	0.6989

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	52.1232	0.7011	0.2989
NESTED CLADE	-1.9349	0.6989	0.3011

PERMUTATION ANALYSIS OF 5-1 ALL SOUTH ISLAND (24)
BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 303.0815

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 4-1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	185.9890	0.9334	0.0666
NESTED CLADE	179.2293	0.7489	0.2511

CLADE 4-2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	49.3332	0.0000	1.0000
NESTED CLADE	116.0554	0.0000	1.0000

CLADE 4-3 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	19.9736	0.0000	1.0000
NESTED CLADE	252.1244	0.9865	0.0135

CLADE 4-4 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	49.7497	0.0002	0.9998
NESTED CLADE	216.5033	0.9087	0.0913

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	-108.2966	0.0074	0.9926

NESTED CLADE	89.2420	0.9905	0.0095
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ERMUTATION ANALYSIS OF 1-28 NORTH ISLAND 1
 BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 6.6667

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
 OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.7033

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE A1a1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	7.7467	0.8007	0.3976
NESTED CLADE	9.2353	0.8007	0.3976

CLADE A2a1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	2.5611	0.2023	1.0000

CLADE A3a1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	15.3315	1.0000	0.4022

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	7.7467	0.8007	0.3976
NESTED CLADE	0.2889	0.8007	0.3976

PERMUTATION ANALYSIS OF 2-16 NORTH ISLAND ALL
 BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 1.2000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
 OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-28 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	6.7928	0.4985	1.0000
NESTED CLADE	7.2464	0.4985	1.0000

CLADE 1-29 (Tip)			
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	14.0710	1.0000	0.4985

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

PERMUTATION ANALYSIS OF 1-30 OTAGO NORTH 1
 BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 8.0294

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
 OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.5791

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE CN1cn1 (Interior)			
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	28.2224	0.2028	0.8102
NESTED CLADE	27.4555	0.2587	0.7543

CLADE CN1cn3 (Tip)			
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.2336	1.0000
NESTED CLADE	41.6413	0.9077	0.2297

CLADE CN2cn1 (Tip)			
TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.2406	1.0000
NESTED CLADE	41.6413	0.9025	0.2328

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	28.2224	0.8457	0.1673
NESTED CLADE	-14.1858	0.1956	0.8174

PERMUTATION ANALYSIS OF 1-31 OTAGO NORTH 2
 BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 3.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
 OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.6737

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE CN1cn2 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	11.6594	0.6599	0.6737
NESTED CLADE	11.7399	0.3336	1.0000

CLADE CN4cn2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	35.8318	1.0000	0.3336

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	11.6594	0.6599	0.6737
NESTED CLADE	-24.0919	0.3336	1.0000

PERMUTATION ANALYSIS OF 1-34 OTAGO SOUTH 1
BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 11.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.1872

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE CS1cs1 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	16.9792	0.0960	1.0000
NESTED CLADE	19.7375	0.0960	1.0000

CLADE CS3cs1 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	0.6364	1.0000
NESTED CLADE	46.9392	1.0000	0.0960

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	16.9792	0.0960	1.0000
NESTED CLADE	-27.2017	0.0960	1.0000

PERMUTATION ANALYSIS OF 1-36 OTAGO SOUTH 2
BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 0.7500

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE CS2cs2 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	5.3569	1.0000	0.6624
NESTED CLADE	4.6874	1.0000	0.6624

CLADE CS4cs2 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	2.1871	0.6624	1.0000

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	5.3569	1.0000	0.6624
NESTED CLADE	2.5003	1.0000	0.6624

PERMUTATION ANALYSIS OF 2-17 OTAGO 3
BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 34.9143

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.1168

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-30 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	28.1575	0.0000	1.0000
NESTED CLADE	58.8966	0.7136	0.2864

CLADE 1-33 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0001	1.0000	0.1513
NESTED CLADE	58.9653	0.6125	0.5388

CLADE 1-34 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	24.2711	0.0039	0.9961
NESTED CLADE	55.8308	0.3601	0.6399

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

PERMUTATION ANALYSIS OF 2-18 OTAGO 4
 BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 1.3333

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
 OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-31 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	14.4168	0.7510	0.7489
NESTED CLADE	14.2829	0.7510	0.7489

CLADE 1-32 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	0.0000	1.0000	1.0000
NESTED CLADE	32.2469	1.0000	0.4999

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	14.4168	0.7510	0.7489
NESTED CLADE	-17.9640	0.4999	1.0000

PERMUTATION ANALYSIS OF 2-19 OTAGO 5
 BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 4.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
 OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.4078

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 1-35 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	6.8033	0.3938	0.7091
NESTED CLADE	17.0512	0.7058	0.3971

CLADE 1-36 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	3.6456	0.1029	1.0000
NESTED CLADE	14.7999	0.2046	0.8983

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	3.1577	0.8983	0.2046
NESTED CLADE	2.2513	0.8983	0.2046

PERMUTATION ANALYSIS OF 3-10 OTAGO ALL
BASED ON 10000 RESAMPLES

PART I. EXACT CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 77.5515

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

CLADE 2-17 (Interior)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	57.5127	0.3634	0.6366
NESTED CLADE	57.5334	0.3124	0.6876

CLADE 2-18 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	16.0793	0.0667	0.9334
NESTED CLADE	35.9937	0.0607	0.9394

CLADE 2-19 (Tip)

TYPE OF DISTANCE	DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	15.9097	0.0222	0.9778
NESTED CLADE	86.5554	0.9578	0.0422

PART III. TEST OF INTERIOR VS. TIP CLADES:

TYPE OF DISTANCE	I-T DISTANCE	PROB.<=	PROB.>=
WITHIN CLADE	41.5276	0.9886	0.0114
NESTED CLADE	-6.5501	0.3141	0.6859