Cover letter
Click here to download Cover letter: Letter to Editor.docx

Editor Conservation Genetics
re manuscript COGE-08-353
May 26 2009

Dear Sir

Thank you for your provisional acceptance and comments from Reviewer 1. We appreciate the input and have made changes accordingly. We believe it is a better manuscript and hope you consider it ready for publication in Conservation Genetics.

Sincerely
Jennifer M. Hay

Genetic diversity and taxonomy: a reassessment of species designation in tuatara (*Sphenodon*: Reptilia)

Jennifer M. Hay, Allan Wilson Centre for Molecular Ecology and Evolution at Institute of Molecular BioSciences, Massey University, PB 102904 NSMC, Auckland, New Zealand.
jenniehay@hotmail.com Phone and Fax: +1 520 398-8040

Stephen D. Sarre, Institute for Applied Ecology, University of Canberra, ACT 2601, Australia

David M. Lambert, Griffith School of Environment, Griffith University, Nathan Campus, 170 Kessels Road, Qld 4111, Australia

Fred W. Allendorf, Division of Biological Sciences, University of Montana, Missoula MT 59812, USA

Charles H. Daugherty, School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

Running title for page headings
Genetic diversity and taxonomy of *Sphenodon* populations.

Keywords
Microsatellite DNA; mitochondrial DNA; allozymes; phylogenetics; taxonomy; conservation

ABSTRACT

The identification of species boundaries for allopatric populations is important for setting conservation priorities and can affect conservation management decisions. Tuatara (*Sphenodon*) are the only living members of the reptile order Sphenodontia and are restricted to islands around New Zealand that are free of introduced mammals. We present new data of microsatellite DNA diversity and substantially increased mtDNA sequence for all 26 sampled tuatara populations. We also re-evaluate existing allozyme data for those populations, and together use them to examine the taxonomic status of those populations. Although one could interpret the data to indicate different taxonomic designations, we conclude that, contrary to current taxonomy, *Sphenodon* is best described as a single species that contains distinctive and important geographic variants. We also examine amounts of genetic variation within populations and discuss the implications of these findings for the conservation management of this iconic taxon.

INTRODUCTION

Determining the taxonomic status of species containing allopatric populations with unique geographic variation and no migration has always been problematic. Mayr (1963) predicted that new species will arise in bottlenecked founder populations isolated from the parent population due to selection and/or genetic drift. Indeed, a common characteristic of archipelagos is closely related species on different islands (e.g., Parent et al. 2008, Whittaker et al. 2008). Taxonomy that accurately reflects the levels of differentiation at or near species level can be difficult under these circumstances, and molecular systematics becomes a fusion of phylogenetics and population genetics. The question becomes more than just academic when considering species with high conservation importance resulting from their unique features or rarity. Management decisions such as mixing or translocating animals from small vulnerable populations may differ if they are seen as distinct species or subspecies, or merely populations with some genetic distinctiveness.

One exemplar of difficult species determination and therefore conservation strategy is the New Zealand reptile, *Sphenodon* (tuatara), the sole remnant of the order Sphenodontia. The ancient sphenodontian lineage was a globally distributed and diverse group that originated in the Triassic and which otherwise expired in the late Cretaceous. Tuatara skeletal features have changed little from some Cretaceous members (Benton 1993; 2000; Apesteguía & Novas 2003). Subfossil bone deposits (<10,000 years old) indicate that tuatara were once found throughout the New Zealand landmass. However, all populations were lost from the mainland (North Island, South Island, Stewart Island) sometime after the arrival of humans and exotic mammals starting ~800 years ago (Duncan et al. 2002; King 2003), leaving only those populations previously isolated on islands formed by post-glacial rising sea-levels 8000-12,000 years ago (Hayward 1986). Today tuatara wild populations are found on 32 islands, plus at least three islands on which tuatara colonies were established from existing populations (Gaze 2001). If left alone with no management at all, it was predicted that many tuatara populations would go extinct because of the small size of the islands and/or small size of the populations (Daugherty et al. 1992). In pre-human times tuatara were a top-level predator and they are naïve to mammalian predators that may inadvertently colonise the islands, e.g., rats (*Rattus rattus, R. norvegicus, R. exulans*), mustelids (stoats, weasels), and feral cats and dogs. Even rabbits and feral pigs can adversely affect the undergrowth and food supply of this burrowing, medium-sized carnivore.

Nineteenth century biologists named a number of species and subspecies of tuatara based on morphological characters. Of particular relevance here, the population on North Brother Island in Cook Strait and the now extinct East Island population were identified as worthy of the species designation *Sphenodon guntheri* separate from *Sphenodon punctatus* (Buller 1877, 1878, 1879) on other islands. Subsequently, twentieth century biologists largely dismissed the various species based on informal observations of colour variation within and between islands, and recognised only *Sphenodon punctatus* (e.g., Dawbin 1962). That single species designation within tuatara was challenged by Daugherty et al. (1990) who found a clear genetic separation between the population on North Brother Island and two other groups (western Cook Strait islands and all northern populations) on the basis of both allozyme genetic and morphometric variation (Fig. 1). As a result of that work, the dual species designation of *S. punctatus* and *S. guntheri* was adopted by the New Zealand Department of Conservation who instigated captive breeding, island restoration including pest eradication, and translocation programmes for all three groups, with much effort directed towards the sole population of *S. guntheri* on North Brother Island (Cree & Butler 1993; Gaze 2001; Nelson et al. 2002a). These measures are effectively securing the future of all three genetic groups (*S. guntheri* "Brothers tuatara",

S. punctatus "western Cook Strait tuatara" and *S. punctatus* "northern tuatara"), and the complete island ecosystems of plants and animals, many of which are also rare or vulnerable.

More recently, Hay et al. (2003; 2004) examined mitochondrial DNA (mtDNA) sequence variation (ND1, partial control region and cytochrome b) among tuatara populations. They recovered the two previously identified groups of *S. punctatus* (northern islands and western Cook Strait islands) but did not find the deep split between *S. guntheri* and *S. punctatus* that was evident in the allozyme data. *Sphenodon guntheri* mtDNA haplotypes grouped together but with other Cook Strait populations (Fig 1 inset). It was proposed that two Evolutionarily Significant Units (ESUs; Ryder 1986; Waples 1991) be recognised within *S. punctatus* representing northern and western Cook Strait populations respectively (Hay et al. 2003). However, the recognition of two ESUs within *S. punctatus* left *S. guntheri* in a compromised position because the mtDNA sequence data place *S. guntheri* paraphyletic to one of the ESUs defined by the mtDNA clades whilst it taxonomically it represents a separate species.

The source of the discrepancy between the mtDNA and allozyme tuatara datasets is unclear (Hay et al. 2003). The two types of genetic datasets have different transmission histories (maternal mtDNA vs. biparental nuclear genes), and different susceptibilities to selection (in protein allozymes) and genetic drift (higher in mtDNA), and give information on different time scales (faster rates of detectable mutation in mtDNA sequence than allozyme amino acid changes). Influences on these analyses from sex-biased dispersal is unlikely given that both male and female juvenile tuatara disperse from the nest site, yet tuatara rarely if ever disperse between islands (demonstrated here and in MacAvoy et al. 2007), and the samples were collected on an adhoc basis from anywhere on each island. To better understand patterns of genetic variation in tuatara populations and the implications for their taxonomy, we chose to examine faster-evolving nuclear microsatellite loci expected to be selectively neutral and contain more variation than allozymes or gene sequences. MacAvoy et al. (2007) employed six microsatellite loci to examine genetic diversity in 14 representative populations of tuatara. This provided much valuable information about those populations, but we were concerned to examine every population (that had been able to be sampled) and to look for patterns within as well as between the 12 groups of islands (Fig. 1). However, this strategy limited the sample size per population able to be genotyped. In addition to genotyping microsatellite DNA we greatly increased the mitochondrial DNA dataset (Hay et al. 2003) by sequencing the complete control region for all 26 sampled populations of tuatara.

Concerns that phylogenetic trees of gene frequency data lose information and can distort genetic relationships among populations (Menozzi et al. 1978; Allendorf & Leary 1988) led us to use principal component analysis (PCA) to reexamine geographic patterns in the existing tuatara allozyme data (Daugherty et al. 1990; Hay et al. 2003) and new microsatellite DNA data. To see if we had overall support (or lack of support) for two or three higher orders of groups of populations (corresponding to the existing mtDNA and allozyme groups) we used AMOVA to test for genetic structure of the three different datasets. We examine genetic variation in tuatara populations in terms of distinctiveness from each other and heterozygosity levels within populations and discuss the implications of the data for taxonomy and tuatara population management.

METHODS

Genomic DNA was purified from blood samples from natural populations (not translocated ones), most collected from 1989 to 1991 (Daugherty et al. 1990; Hay et al. 2003) and stored at –80ºC by CHD as part of the National Frozen Tissue Collection. The samples used for microsatellites and mtDNA are a subset of those used for the allozyme study (Daugherty et al. 1990) except for the Hen and Chickens Islands samples which were collected by DML in the mid-1990s and are stored at –80ºC by DML. Samples used for each data set are given in the online Supplementary Material.

DNA extraction, microsatellite DNA PCR and genotyping methods used were those described in Aitken et al. (2001) to examine variation at five tuatara-specific microsatellite DNA loci (1/A6, 1/B3, 1/C1, 1/C2, 2/A12). A sixth locus (1/C12) from Aitken et al. (2001) was not used because unambiguous allele identification was difficult in some individuals. Any scores with ambiguity were repeated until we were confident of the genotype. Multiple samples covering the range of allele sizes for each locus were repeated each run to ensure consistent scoring. In total, we genotyped 144 individual tuatara from 26 populations and all 12 island groups (Fig. 1). Although our aim was to genotype six individuals per population, fewer than six samples were available from some tiny populations on islands <1ha. As with the few wild populations that could not be sampled, these small rock outcrops are part of island groups with larger islands and tuatara populations which were sampled. We genotyped seven tuatara from Stephens Island and 18 from the single natural population of *S. guntheri* on North Brother Island.

We chose to sequence the mitochondrial DNA control region because it has more variation than the ND1 and cytochrome b genes sequenced previously by Hay et al. (2003; 2004). Amplification of the control region by PCR followed the protocols of Hay et al. (2003) using the primers ProL and PheTH from Table 2 of that paper. These primers were also used for sequencing both strands. Where necessary to sequence through homopolymeric runs, internal primers described by Hay et al. (2003) were used. In tuatara, a portion of their control region is duplicated and lies between glutamate tRNA and leucine tRNA (Rest et al. 2003). To ensure the correct control region was amplified and sequenced for the present study at least one primer in each PCR was located outside the duplicated region. Sequencing was conducted by the Massey University sequencing facilities in Palmerston North and Auckland. In total we sequenced partial proline tRNA, complete serine tRNA, complete control region, and partial phenylalanine tRNA of 106 individual tuatara from all 26 populations. Mitochondrial sequences are presented on the light strand.

Microsatellite DNA analyses

To ensure the microsatellite loci are independently inherited we tested each pair of loci for linkage disequilibrium using the pairwise linkage test in Arlequin 3.01 (Excoffier et al. 2005). To examine microsatellite diversity within tuatara populations we calculated standard population genetics estimates (allele frequencies and heterozygosities (H_M)) using GENEPOP ver. 3.2 software (Raymond & Rousset 1995). Populations were tested for Hardy-Weinberg equilibrium at each locus using Arlequin 3.01. To further examine amounts of variation within populations, heterozygosity estimates were plotted against both log island sizes (in hectares) and log population sizes. One would expect heterozygosities to be higher in larger populations and therefore on larger islands (Frankham 1996). These were tested with regression statistics calculated with Matlab 7.4.0.287 (R2007a, The Mathworks Inc. Natick, MA). Island sizes and population size estimates were taken from Cree and Butler (1993), updated in Gaze (2001). These population size estimates are necessarily rough because of the difficulty of obtaining mark-recapture data from this number of remote populations, some of which were visited for just a few

hours. For the purpose of specifying a number on our analysis and to retain relative population sizes, we converted estimates of '10s' to '50' , 'few 10s' to '30', 'few 100s' to '300', '100s' to '500', and 'few 1000s' to '3000'. 'High 100s to low 1000s' became '1000'. The North Brother Island population size has been estimated with greater precision to be 450 tuatara (Nelson et al. 2002b).

Most islands separated from the mainland only 8000-12,000 years ago (Hayward 1986), and tuatara have long generation times (50 years, Allendorf and Luikart 2007). To see whether the microsatellite loci contained sufficient signal to discriminate populations, or whether the signal was obscured by shared ancestry of haplotypes or homoplasy of number of repeats, log likelihood G statistics testing for population differentiation were calculated using FSTAT (Goudet et al. 1996; Goudet 2001). We used sequential Bonferroni corrections for multiple non-independent pairwise comparisons (Rice 1989). Similarly, population assignment tests were conducted using Arlequin 2.0 (Schneider et al. 2000) likelihood estimates of each individual genotype occurring in all populations, based on population allele frequencies (Paetkau et al. 1995; 1997; Waser & Strobeck 1998).

To determine whether the microsatellite variation supported three groups of populations as defined by the previous allozyme analysis (northern, western Cook Strait and North Brother Island, Daugherty et al. 1990) or two as defined by the mtDNA (northern and all Cook Strait islands, Hay et al. 2003), we conducted hierarchical analysis of molecular variance tests (AMOVA Excoffier et al. 1992) testing both population structures using Arlequin 3.01. We used number of different alleles (FST) distance estimates rather than sum of squared size differences (RST) to do this because of the small sample sizes per population (Gaggiotti et al. 1999). Thus, these tests use fixation indices to calculate how much of the genetic variation falls within and between populations and groups of populations.

It has been suggested that the North Brother population in particular has undergone bottlenecks (Hay et al. 2003; MacAvoy et al. 2007). Small sample sizes prevented meaningful quantitative tests for bottlenecks (Cornuet and Luikart 1996, Luikart and Cornuet 1998, Luikart et al. 1998a, 1998b, Piry et al. 1999) because there are too few variable loci in each population to overcome stochastic differences. The numbers of polymorphic loci and of alleles per locus have been found to be more sensitive indicators of bottlenecked populations (Leberg 1992) so these were compared in populations on similarly sized islands.

Principal components analysis (PCA) of the microsatellite data was conducted using MINITAB (release version 13.31), to compare with the reanalyses of the allozyme data (see below).

To evaluate the utility of the microsatellites for tuatara phylogenetic analysis, a minimum evolution tree of individuals as well as populations (not shown) was constructed in MEGA 3.1 (Kumar et al. 2004) using Nei's D_a distance (Nei et al. 1983) calculated with MSA 4.05 (Dieringer & Schlötterer 2003). Although Goldstein et al's (1995) $(\delta\mu)^2$ distance is designed on the stepwise mutation model specifically for microsatellites, it is not as good for closely related populations (Goldstein et al. 1995) and indeed gave less resolution than other distances (results not shown) so was not used. Nei's standard distance D (Nei 1978), Cavalli-Sforza and Edwards (1967) chord distance D_c and proportion of shared alleles distance D_{pc} (Bowcock et al. 1994) were also used and gave similar but no more information than Nei's D_a (results not shown).

Mitochondrial DNA analyses

Nucleotide sequences were compiled and aligned in Sequencher ver. 4.1 (Gene Codes Corporation) and checked by eye. The mean genetic distance between populations and island groups was estimated with MEGA 3.1 (Kumar et al. 2004) using Kimura-2 distances and the pairwise deletion option for treatment of gaps (i.e., gap sites were used in pairwise comparisons except when there was a gap in one or both sequences). Both tree and network analyses of the mtDNA were conducted. Kimura-2 distances were used to construct minimum evolution trees in MEGA 3.1 using the pairwise deletion option and the topology was tested with confidence probabilities (CP) and bootstrap confidence levels (BCL) with 1000 replications. The root for the phylogenetic tree was set between northern and Cook Strait populations in accordance with the mitochondrial genome root position identified by Hay *et al.* (2004) which used mitochondrial cytochrome b and an ancient nuclear copy of cytochrome b; there is no living animal related closely enough to act as an outgroup taxon. Parsimony trees of mtDNA haplotypes were found using PHYLIP ver. 3.67 (Felsenstein 2005) with a heuristic search and a strict consensus tree made of the equally most parsimonious trees. PHYLIP treats gaps as a 5^{th} character. The dataset was bootstrapped 100x and a majority rule consensus tree made of the results to test the parsimony tree. A maximum likelihood (ML) tree was constructed with PAUP* ver.4.10 beta (Swofford 2003) using the best fit model found under the Akaike Information Criteria in Modeltest 3.5 (Posada and Crandall 1998), which equated to the general time reversible model utilising data base frequencies (f[A]=0.3206, f[C]=0.2185, f[G]=0.1151, f[T]=0.3458), relative rates of transitions (13.0025) and both transversions (Watson-Crick pairs 2.5919, nonWatson-Crick pairs 1.0000), proportion of invariable sites (0.8874) and gamma parameter (α=0.9154). PAUP cannot treat gaps as a 5^{th} character for ML analyses so gap information was not used in this analysis. Median joining networks (Bandelt et al. 1999) were constructed using NETWORK 4.111 software (http://www.fluxus-engineering.com). To compare genetic structure with the microsatellite DNA data we conducted the same AMOVA tests on mtDNA with three and then two groups of populations (see explanation above).

Allozyme reanalyses

We performed principal components analysis (PCA) on the allozyme data of Daugherty et al. (1990) and Hay et al. (2003) using MINITAB (release version 13.31). We computed the PC scores based on the covariance matrix of allele frequencies at all 12 variable loci. The second most frequent allele at each locus was omitted to account for the non-independence of allele frequencies within each locus (Reich et al. 2008).

Allozyme heterozygosities (H_A) were available from Hay et al. (2003), but were re-estimated here with GenePop to ensure we were making direct comparisons with those of the microsatellites. Also, to make comparisons between the microsatellite and mtDNA analyses, we tested for signals of genetic structure of three groups (allozyme-defined) and two groups (mtDNA and geographically-defined) of islands using AMOVA in Arlequin. Additionally we tested for signals of recent bottlenecks under the infinite alleles model using BOTTLENECK (Piry et al. 1999) which looks for evidence of deficiency (or excess) of heterozygotes. This test was possible for allozymes but not for microsatellites because allozyme sample sizes (293 individuals total) were higher than for microsatellites. As for microsatellites, numbers of polymorphic loci and alleles per locus were compared.

RESULTS

Characteristics of mitochondrial DNA

Hay et al. (2003) examined 12 tuatara individuals for a total of 678 bp control region and 570 bp NADH1 sequence. Here we increased the sample size to 106 tuatara for 1039 bp complete control region and partial flanking tRNAs (Genbank Accession numbers xxxxx-yyyyy). The control region is 924-929 bp long with most length differences due to lengths of homonucleotide runs. The 106 sequences reduce to 50 haplotypes. The 106 sequence dataset has 48 variable sites (75 including gap sites), 43 of which are parsimony informative (64 including gaps). Interpopulation Kimura-2 distances ranged from 0-0.02; the highest values were between Brothers and some Aldermen, Mercuries and Cuvier animals (Table 1).

Characteristics of microsatellite loci

All of the microsatellite DNA loci were polymorphic with 8-26 alleles per locus (Table 2). There was no evidence of linkage disequilibrium between the five microsatellite loci. The only evidence for null alleles was in the Brothers population in the otherwise monomorphic locus 1/C1, where two individuals consistently gave no result at that locus despite repeated attempts and working well for the other loci. Only the North Brother Island sample was large enough to test for Hardy-Weinberg equilibrium (HWE): 2 loci were monomorphic (1/A6 and 1/C2), one was in HWE (2/A12 P=0.314), one had a deficiency of heterozygotes (1/B3 P=0.002), and one had apparent heterozygote deficiency (1/C1 P=0.003) probably due to null alleles.

The sample sizes used here are too small to capture all rare alleles present, but are large enough to detect common alleles. If an allele has a frequency of p, each time we examine one gene there is a probability of p of detecting it and a probability of $(1-p)$ of not detecting it. The probability of not detecting an allele at a frequency p in a sample of 6 individuals ($2 \times 6 = 12$ alleles) is $(1-p)^{12}$. We therefore have a greater than 95% probability of detecting an allele with frequency of p=0.22 in a random sample of 6 individuals. There are a few alleles private to populations or island groups (Supplementary Material). Some may be artefacts of small sample sizes, but six occur at higher than p=0.22 (Red Mercury, Cuvier and two each in Coppermine and Moutoki), which suggests that if they existed in other populations they would have been detected. North Brother has no private alleles except possibibly the null allele in locus 1/C1, although the null may be hidden in other more variable populations.

Genetic variation within populations

North Brother, Moutoki Hongiora and Hernia exhibited no intrapopulation mtDNA variation, whereas the rest contained mean within-population distances of 0.002-0.01 (Table 1). A wide range of expected heterozygosities at microsatellite loci (H_M) was found in population samples (0.13-0.80). Regression analyses revealed a strong association between H_M and the log of island size ($R^2 = 0.414$, $P < 0.001$; Fig. 2). However, there is no relationship between the log of current estimated population size and H_M ($R^2 = 0.037$, $P = 0.348$) or H_A ($R^2 = 0.003$, $P = 0.779$), or of H_A to log island size ($R^2 = 0.077$, $P = 0.171$). Correspondingly, present log population size is not correlated with log island size (log $R^2 = 0.011$, $P = 0.608$). There is high microsatellite genetic diversity (Table 2) in the four small remnant populations on large islands, Little Barrier ($H_M = 0.68$), Cuvier ($H_M = 0.58$), Red Mercury ($H_M = 0.67$) and Stanley ($H_M = 0.80$). These levels are comparable to those on Stephens Island in Cook Strait ($H_M = 0.66$), which is also a large island but contains a large tuatara population (150 ha, ~30,000 tuatara).

There is approximately ten-fold greater heterozygosity at microsatellite loci than allozymes because of the faster mutation rates of microsatellite loci (Tables 2 and 3). A number of populations have

relatively low H_A but high H_M, e.g., Hen, Little Barrier and Karewa (Table 2 and 3). Overall there is no statistical correlation between H_M and H_A ($R^2 = 0.101$, $P = 0.113$). Similarly, microsatellites contain much more variation than mtDNA, where some groups with high H_M have no mtDNA diversity (e.g., Little Barrier). BOTTLENECK tests (Piry et al. 1999) of the allozyme data gave no evidence of heterozygosity excess or deficiency with respect to mutation-drift equilibrium (results not shown). North Brother tuatara have low microsatellite number of alleles (n_A) and polymorphic loci (n_P) and low mtDNA variation and allozyme H_A, but medium allozyme n_A and n_P. Poor Knights have low n_A and n_P for both microsatellite and allozymes, but fall into two groups of northern tuatara mtDNA haplotypes. Moutoki has low microsatellite and mtDNA variation, but not allozyme. Karewa, Motunau and Trios Islands have low allozyme n_A, n_P and H_A and low mtDNA diversity, but this is not reflected in their microsatellite n_A, n_P and H_M.

Genetic structure among populations

There is sufficient signal in the microsatellite to distinguish populations despite the small sample sizes. The log-likelihood G values (Goudet et al. 1996) for the microsatellite DNA data show that this dataset has much higher than random probabilities of tuatara population differentiation. From the assignment tests, likelihood estimates of individual genotypes in all populations were successful in assigning 93.9% individual tuatara to their correct population (Table 4). Thus, 18 of 26 populations could be unambiguously distinguished from all other populations with these microsatellite DNA loci. This increased to 95.7% of individual tuatara correctly assigned when populations were combined into their island groups (Table 5).

The principal component analysis of the microsatellite data (Fig 3a) gave low values of percent of the variance in allele frequencies, but with similar patterns to that of the assignment test and microsatellite tree (see below). The first principal component (31.3%) separates the Poor Knights islands, and North Brother and Moutoki from the remaining populations of both northern and Cook Strait islands. The second principal component (13.3%) separates Moutoki and North Brother from all other populations. These PCA-discriminated populations also have the lowest microsatellite heterozygosities, number of alleles and polymorphic loci whereas the other populations shared more alleles.

In contrast, the PCA analysis of the allozyme data reveals that both patterns of metapopulation structure are apparent in the plot of the first two components (Figure 3b). The first component (49.5% of the variation) distinguishes between the Northern group and the Cook Strait populations such that the North Brother population clusters closely with the Western Cook Strait populations on this axis. The second principal component (34.3%) separates the North Brother population from the other 25 populations. These results mirror both of the population structures as seen in the AMOVA tests (below) but show the mtDNA pattern more strongly than the phylogenetic allozyme pattern.

The mtDNA minimum evolution tree (Fig. 4) and median joining network (Fig. 5) for 106 individuals show clearly the separation of northern and all Cook Strait tuatara (99% CP, 98% BCL). Within Cook Strait, North Brother tuatara form a tight group (96% CP, 96% BCL). The structure of the northern tuatara partially follows island groups but with some mixing. As seen in microsatellite analyses, Moutoki form a distinct group at 99% CP (99% BCL). One group of Poor Knights tuatara cluster together at 98% CP (99% BCL), and the remainder of the northern tuatara form two groups (97% CP/96% BCL and 86% CP/65% BCL respectively) with some populations from Hen and Chickens, Aldermen and Karewa represented in both genetic clusters. Parsimony analysis found 24 most

parsimonious trees of 131 steps for 50 haplotypes (Fig. 6). The strongest bootstrap supported node was 100% between all northern and all Cook Strait populations. Likelihood analyses found two ML trees with –Ln likelihood score of 1889 (see Supplementary Material).

The microsatellite tree of individuals (Fig. 7) shows less population structure than the mtDNA tree and reflects the microsatellite PCA and assignment test results, i.e., North Brother tuatara cluster together, as do Poor Knights tuatara and Moutoki. The analysis does not reveal a clear separation of northern and Cook Strait populations as seen in mtDNA and allozyme trees.

AMOVA analyses were used on microsatellite, allozyme and mtDNA data sets to test the genetic structure of the populations (Table 6). We tested for partitioning of the populations into two groups (northern and Cook Strait, as per mtDNA patterns [Hay et al. 2003]) and into three groups (northern, western Cook Strait and Brothers, as per allozyme patterns [Daugherty et al. 1990]). Genetic variation in all three datasets was found to be consistent with both two and three group hierarchies at $P \leq 0.05$ (Table 6), i.e., neither of the genetic-geographic structure hypotheses was rejected with any dataset. Over half of the total allozymes and mtDNA variation observed is attributable to inter-group differences compared with only 5-8% of the variation among microsatellite loci, indicating that most microsatellite DNA variation is contained within populations. Accordingly, F_{ST} estimates are much higher for mtDNA and allozymes than for microsatellites (data not shown).

DISCUSSION

Genetic diversity, taxonomy and management of tuatara for conservation

Sphenodon are iconic and are of cultural and scientific value to indigenous and non-indigenous New Zealanders and the international community. As a consequence, and because they have clearly declined in distribution and number over recent centuries (Daugherty et al. 1992), tuatara are the focus of sustained conservation effort. The New Zealand Department of Conservation first instigated a recovery plan specifically for tuatara in 1993 (Cree & Butler 1993), which was updated in 2001 with a ten year plan that includes captive breeding and the establishment of populations through translocations to predator-free islands (Gaze 2001). One goal of the tuatara recovery plan is the maintenance of genetic diversity among tuatara. As such, a key research priority in the recovery plan is, 'to better understand taxonomic relationships among tuatara, including confirmation of the subspecific status for tuatara in Cook Strait [i.e., Cook Strait vs. northern *S. punctatus*]' (Gaze 2001, p. 26). Gaze notes that the understanding of this relationship has direct relevance to the allocation of resources for the conservation of tuatara and in determining the desirability of translocations among specific islands. In particular, the New Zealand Department of Conservation recognised *S. guntheri* (Brothers tuatara) as a category A species (requiring urgent recovery work) but not the Cook Strait *S. punctatus* and northern *S. punctatus* (which they recognised as a category B species - requiring work in the short term). These species recognitions were made largely on the basis of the allozyme and morphometric differences observed by Daugherty et al. (1990).

The extant tuatara populations, which are all on islands, were separated from the mainland populations 8000-12,000 years ago and the genetic differences between the groups will reflect previous genetic patterns, recent independent evolution or a combination of both. We address the relative contributions of these factors elsewhere using ancient DNA of mainland populations (Fig 1c of Subramanian et al. 2008; Hay and Lambert unpubl. data) but note that those data do not contradict the conclusions

presented here. We note also that island populations of squamates, when isolated for a similar period of time by rising sea levels, exhibit similar or greater levels of genetic divergence in allozymes and mtDNA among populations (e.g. Soule 1973; Sarre et al. 1990; Brehm et al. 2003; Hanley & Caccone 2005; Keogh et al. 2005; Terrasa 2009).

In the case of tuatara, all three datasets analysed here, allozyme, mtDNA and microsatellite, suggest support for the distinctiveness of North Brother tuatara, but the evidence is equivocal. First, it must be noted that the original allozyme based separation of *S.guntheri* from other tuatara (Daugherty et al. 1990; Hay et al. 2003) was not based merely on "a single fixed difference" as cited in MacAvoy et al. (2007). Of the nine variable allozyme loci, two loci (one fixed and one nearly fixed) differentiate Brothers tuatara from all other populations, and two variable loci separate Brothers from other Cook Strait and from northern populations respectively. These differences resulted in much higher genetic distances between Brothers tuatara and all other populations, than all pairwise distances among other populations. In addition, discriminant function analysis of seven morphometric characters clearly separated North Brother, western Cook Strait and northern populations with 66-86% accuracy (Daugherty et al. 1990). In the present study, AMOVA tests show all three genetic datasets are consistent with an overall structure of either three or two groups of populations, corresponding with both the original allozyme and mtDNA trees respectively (Fig. 1 insert). In PCA (Fig. 3) and tree analyses (Fig. 7) of the microsatellite data Brothers tuatara do form a distinct group. MacAvoy et al. (2007) found the same separation of Brothers tuatara from all other populations in their PCA of pairwise population F_{ST} estimators (θ). Phylogenetic analyses of the greatly expanded mtDNA dataset (Figs. 4-6) significantly separate northern from Cook Strait populations, and Brothers tuatara form a distinct group, although within the Cook Strait group. Brothers tuatara have the highest pairwise mtDNA distances to all other populations (Table 1). However, the mtDNA tree differs from the original allozyme tree which showed the North Brother population to be highly divergent from and equidistant to all other populations (Daugherty et al. 1990; Hay et al. 2003). Reanalysis of the allozyme data using principal component analysis reveals a slightly different interpretation than that suggested by the original distance-based tree analyses. The primary division (49.5%) identified by the allozyme principal component analysis (Fig. 3b) is a split between all Cook Strait islands and all northern populations, which is the predominant pattern seen in the mtDNA analyses, followed by a second order split (34.3%) between Brothers tuatara and all other populations (Cook Strait and northern) as per allozyme genetic patterns.

In the present study, the North Brother tuatara population has the lowest microsatellite DNA heterozygosity and number of polymorphic loci and alleles/locus of any of the tuatara population samples – substantially lower than other similarly small populations on small islands (Green Mercury, Hernia, Motunau and Moutoki; Table 2). The 100% assignment accuracy for North Brother individuals is probably owing to the modest microsatellite DNA variation among Brothers tuatara, which results in multiple identical composite genotypes (only seven genotypes were recovered in 18 animals); no other animals in this study share microsatellite genotypes. MacAvoy et al. (2007) found even less microsatellite DNA variation in Brothers tuatara, with five of their six loci monomorphic. They also detected a mode shift in their Brothers tuatara allele frequency spectrum, which they interpret as due to a historical bottleneck, probably a founder effect. It is the very lack of variation in Brothers tuatara microsatellite data that prohibits most standard bottleneck tests. But the comparatively low microsatellite variation and no within population mtDNA variation (except for one heteroplasmic or ambiguous site in three individuals) is suggestive of inbreeding which could be due to founder effect, population bottleneck (possibly during lighthouse construction or overcollecting, Hay et al. 2003), or

prolonged small population size of the North Brother population. Tests for bottlenecks in the allozyme data gave no evidence of bottleneck events and the North Brother population does not have the lowest allozyme heterozygosity; other larger populations have a lower H_A (Middle Trio, Karewa, Hen; Table 3). The current population of Brothers tuatara is approximately 450 adults, but with 63% males (Nelson *et al.* 2002b) and unequal mating contributions in tuatara (Finch and Lambert 1996; Moore 2008) the effective population size is likely to be much lower. Genetic drift in small populations can allow rare nuclear or mitochondrial DNA alleles to predominate or become fixed in the population (Wilson *et al.* 1985). One could use this argument to dismiss *S. guntheri* as a species and call it an inbred-induced variant. However, one also could use it to argue for species status of *S. guntheri* as this is one way allopatric speciation occurs; geographic isolation and subsequent differentiation of founder populations via genetic drift eventually giving rise to separate species (Mayr 1963, Paterson 2005). Two other groups exhibit reduced genetic variation, although not as extreme as Brothers tuatara, and may have been subject to bottleneck pressures. Moutoki and the Poor Knights populations have low H_M and H_A and number of polymorphic loci and alleles, and are separated out in PCA analyses. Moutoki tuatara additionally have no mtDNA sequence diversity, and are separated out in tree analyses (Tables 2 and 3). If one accepts *Sphenodon guntheri* for the North Brother population based on genetic distinctiveness, one also needs to consider if these other distinctive populations are deserving of species or ESU status.

Overall, the two groups of tuatara most consistently supported by the genetic data are Cook Strait and the northern populations of tuatara. These groups are reciprocally monophyletic and show significant divergence at nuclear loci. The support for a further division of Cook Strait tuatara into *S. guntheri* and *S. punctatus* could only be justified phylogenetically if *S. punctatus* itself is split into two species representing the phylogenetic split between all Cook Strait tuatara and the Northern tuatara. Taxonomic alternatives for tuatara include, 1) one species encompassing all populations with two or three ESUs, 2) two species (northern and Cook Strait), potentially with Cook Strait encompassing two ESUs, and 3) three species (northern, western Cook Strait and Brothers).

Management of populations separately is more easily understood and justified by taxonomic epithets than by tables of data or figures showing genetic distinction. However, two decades of the collection and interpretation of genetic data have led today's conservation managers to be more sophisticated and experienced in their understanding and application of those data (Gaze 2001). Based on the current data, we view tuatara as a single species (*Sphenodon punctatus*), within which three groups: North Brother Island, other Cook Strait islands, and all northern populations are the most consistently observable divisions, whatever the causes (e.g., extinction of intermediate mainland populations, lineage sorting, drift, and/or selection). Note this is not a formal taxonomic description as type specimens were not available for genetic examination and we have not conducted a morphological examination and description. At a practical level, we consider that the initial recommendations of conserving the three lineages of tuatara (Cree & Butler 1993; Gaze 2001) still stand as the most appropriate approach for their conservation. Brothers tuatara are now relatively secure as a result of the successful conservation attention of the past 15 years including multiple new populations. Western Cook Strait tuatara contain the largest population, Stephens Island, and also have increased safety through new translocated populations (Gaze 2001). From the viewpoint of maintaining genetic diversity of all tuatara populations we suggest that a higher priority should now be placed on ensuring the future of the relatively diverse northern populations. Within that group, Poor Knights and Moutoki

should be recognised as containing some unique and important genetic diversity (Figs. 3-7, Tables 2 and 3).

Conservation management tries to balance many factors, such as maximising population genetic diversity while maintaining the purity of local genetic strains which may contain locally beneficial adaptations that would be diluted by mixing with other populations. Without knowing if there is strong local adaptation it is hard to prioritize maintaining local genetic purity, and common sense must always pre-empt narrow genetic guidelines. Many other factors may assume greater importance in management, such as population size, island size, or local environmental conditions.

Without trying to dictate policy, we suggest that northern and Cook Strait tuatara should be kept separate (Gaze 2001), based both on genetic distinctiveness and environmental conditions since the Cook Strait islands experience a cooler climate. Mixing individuals among islands within an island groups seems appropriate as they were separated more recently from each other (<8000 years) than from the mainland and there may be some natural movement among the islands within a group. Although there is no hard evidence for local migration, it seems unlikely that rocky islets <1ha with little vegetation or soil could have maintained their own populations for 8000 years. On a larger scale, if it ever seems necessary to mix populations from different island groups (even North Brother, [Allendorf 2001]), this study shows amounts of variation within and between populations for informed decisions to be made on which populations to mix.

Comparative genetic diversity within tuatara populations

In general, genetic diversity in populations is correlated with population size and length of isolation. Most tuatara island groups have been naturally isolated since the islands formed by rising sea levels 8,000-12,000 years ago. Not surprisingly, small tuatara populations on small islands have lower heterozygosity than larger populations on larger islands (Tables 2 and 3), due to greater genetic drift and inbreeding effects of small populations. Despite this, there is no overall correlation of population microsatellite heterozygosity with tuatara population size, although there is a significant correlation between heterozygosity and island size ($P<0.001$; Fig. 2). We suggest island size indicates long-term population sizes before the impact of humans and commensals which began in New Zealand ~800 years ago; human colonization of onshore islands occurred later than the mainland. The long life-span of tuatara of at least 70 years (Nelson et al. 2002b) means that several decades or perhaps even centuries may be required before declines in population size are reflected in the levels of genetic variation. The strong association between microsatellite heterozygosity and island size supports the notion that tuatara on these islands are largely reproductively isolated, because significant gene flow would destroy this relationship.

In particular, there is high microsatellite DNA diversity indicating historically larger populations of tuatara in the four small remnant northern *Sphenodon punctatus* populations on large islands that had Pacific rats (*Rattus exulans*) and other exotic mammals present (Daugherty et al. 1992): Little Barrier (3083 ha, $H_M=0.68$), Cuvier (170 ha, $H_M=0.58$), Red Mercury (225 ha, $H_M=0.67$) and Stanley (99.5 ha, $H_M=0.80$). These amounts of genetic variation are comparable to those of Cook Strait *S. punctatus* on another large island, Stephens Island (150 ha, $H_M=0.66$) which is the largest population of approximately 30,000 tuatara (Tables 2 and 3). Little Barrier, Cuvier and Red Mercury also contain some private alleles (Supplementary Material). Note that our average expected heterozygosity values for Little Barrier and Stanley are considerably higher than we calculate found by MacAvoy et al.

(2007) (0.45 and 0.46 respectively). As a specific example, in the Mercury Group, the small relictual tuatara populations on relatively large Stanley and Red Mercury islands have higher H_M and H_A than the much larger populations on the smaller Middle and Green Mercury islands (Tables 2 and 3). At each locus, the Stanley or Red populations have microsatellite alleles that are not found in this sample from Middle and Green, and Middle Mercury has some alleles not observed in the other Mercury Islands tuatara populations (Supplementary Material). Before the pest removal, and tuatara population and island restoration programmes were initiated, one option was to allow the Red and Stanley tuatara populations to dwindle away without intervention because the remnant populations were too small and there were healthy populations on other islands in the island group. Instead, the introduced mammals were removed and the habitats are being restored (Gaze 2001). The few Red and Stanley adults are being successful captive bred and their juveniles raised for re-release back on their island of origin. Our study shows this will increase the overall genetic diversity of the Mercury Islands tuatara, and similarly for equivalent programmes underway for Cuvier and Little Barrier tuatara. Little genetic diversity should be lost if a population bottleneck is brief (cf. generation time) and population size recovers rapidly (Lande 1999). The large sizes of these four islands mean they could each potentially sustain several thousands of tuatara. These large populations will be less vulnerable to deleterious inbreeding effects or to extinction by accidental introduction of rats or mustelids (Newman 1986; Daugherty et al. 1992). Already tuatara juveniles of Little Barrier adults captive bred in situ have been genotyped (Moore et al. 2008) and access of particular males to females is being controlled to maximise genetic diversity of offspring.

Similarly, on the Hen and Chickens Islands tuatara currently are found in low numbers, yet they have maintained high microsatellite DNA heterozygosities (H_M=0.65-0.70; Table 2), again suggesting previous larger populations. Pacific rats and cattle have been removed from these islands and we are hopeful for the future of tuatara and other native species there.

Conversely, current larger population size does not necessarily indicate higher population heterozygosity in tuatara. The Poor Knights Islands tuatara contain lower microsatellite heterozygosity (0.17-0.41) than most other tuatara populations, despite relatively large populations on the two larger islands with good habitat, Aorangi and Tawhiti Rahi (~1000 tuatara each, 163 and 110 ha respectively). There may be both human-induced and natural reasons for the lower H_M. As many as 300-400 Mäori resided and cultivated crops extensively on Aorangi and Tahiti Rahi for many generations until the 1920s with occasional extensive fires, and pigs left by Captain Cook in 1769 inhabited Aorangi until 1936 (Fraser 1925; Whitaker 1968). These prolonged habitations may have reduced tuatara population size for an extended period and thereby reduced heterozygosity. The low microsatellite heterozygosity may also reflect the comparatively longer separation of the Poor Knights Islands from the mainland of as long as two million years (Hayward 1991), although they may have been connected for short periods during the last 730,000 years, and possibly as recently as 18,000 years ago (Brook & McArdle 1999). The more recent connection of islands within the Poor Knights group ~8,000 years ago (Brook & McArdle 1999) or rare over-water migration is seen in the strong genetic similarity among their tuatara populations. The longer separation from mainland populations would have heightened the genetic drift effect in rapidly evolving microsatellite DNA, but at slower evolving allozyme and mtDNA loci these Poor Knights tuatara are no more genetically divergent than any other northern population (Daugherty et al. 1990; Hay et al. 2003).

Provenance of tuatara

High amounts of microsatellite diversity within tuatara populations in general resulted in a high probability of assignment of individual tuatara to their correct population (Tables 4 and 5), even using a small number of loci. This has two implications. First it corroborates the notion that overall there was little if any migration of tuatara among islands after island separation from the mainland, whether natural or human mediated. Second, these microsatellite DNA and mtDNA datasets act as useful baselines of background genetic variation of tuatara on all islands with which to compare and identify the provenance of any unknown individuals. This will be of use in identifying poorly labelled museum specimens, old zoo inhabitants around the world, or stolen and smuggled tuatara – a problem for this unique and sought after reptile (e.g., Anonymous 1987).

Conclusion

It is clear that patterns of genetic variation and diversity in tuatara populations are complex and do not always match expectations based on demographic information and island biogeography theory. Some populations with high microsatellite diversity have low mtDNA or allozyme diversity. Some small populations have unexpected high genetic diversity, and some larger populations have lower diversity. With this and our previous studies we have established a strong database of all sampled tuatara populations as a baseline for future studies focussed on specific island populations and for management. The question of species designation in tuatara is no longer as simple as once thought based on clear allozyme differences alone. Without conducting formal species descriptions here, it now seems most appropriate to consider tuatara as a single species, *S. punctatus*, that contains distinctive and important geographic variation.

REFERENCES

Aitken, N, Hay JM, Sarre SD et al. (2001) Microsatellite DNA markers for tuatara (*Sphenodon* spp.). Conserv Genet 2:183-185

Allendorf FW (2001) Genetics and viability of insular populations of reptiles. NZ J Zool. 28:361

Allendorf FW, Leary RF (1988) Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conserv Biol 2:170-184

Allendorf FW, Luikart G (2007) Conservation and the genetics of populations. Blackwell Publishing, Malden, USA

Anonymous (1987) Tuatara traded for drugs. Oryx 21:125

Apesteguía S, Novas FE (2003) Large Cretaceous sphenodontian from Patagonia provides insight into lepidosaur evolution in Gondwana. Nature 425:609-612

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37-48

Benton MJ (1993) The Fossil Record 2. Chapman & Hall, London

Benton MJ (2000) Vertebrate Palaeontology, 2nd edn. Blackwell Science, Oxford

Bowcock AM, Ruíz-Linares A, Tomfohrde J et al. (1994) High resolution human evolutionary trees with polymorphic microsatellites. Nature 368:455-457

Brehm A, Harris DJ, Alves C, Jesus J, Thomarat F, Vicente L (2003) Structure and evolution of the mitochondrial DNA complete control region in the lizard *Lacerta dugesii* (Lacertidae, Sauria). J Mol Evol 56:46-53

Brook FJ, McArdle BH (1999) Morphological variation, biogeography and local extinction of the northern New Zealand landsnail *Placostylus hongii* (Gastropoda: Bulimulidae). J R Soc NZ 29:407-434

Buller WL (1877) Notes on the tuatara lizard (*Sphenodon punctatum*), with a description of a supposed new species. Trans Proc NZ Inst 1876 9:317-325

Buller WL (1878) Notice of a new variety of tuatara lizard (*Sphenodon*) from East Cape Island. Trans Proc NZ Inst 1877 10:220-221

Buller WL (1879) Further notes on the habits of the tuatara lizard. Trans Proc NZ Inst 1878 11:349-351

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. Am J Hum Genet 19:233-257

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014

Cree A, Butler D (1993) Tuatara recovery plan (*Sphenodon* spp.). Threatened Species Recovery Plan Series No. 9. New Zealand Department of Conservation, Wellington

Daugherty CH, Cree A, Hay JM, Thompson MB (1990) Neglected taxonomy and continuing extinctions of tuatara (*Sphenodon*). Nature 347:177-179

Daugherty CH, Towns DR, Cree A, Hay JM (1992) The roles of legal protection versus intervention in conserving the New Zealand tuatara, *Sphenodon*. Developments in Landscape Management and Urban Planning 7:247-259

Dawbin WH (1962) The tuatara in its natural habitat. Endeavour 21:16-24

Dieringer D, Schlötterer C (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Mol Ecol Notes 3:167-169

Duncan RP, Blackburn TM, Worthy TH (2002) Prehistoric bird extinctions and human hunting. Proc R Soc Lond B Biol Sci 269:517-521

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol Bioinform Online 1:47-50

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479-491

Felsenstein J (2005) PHYLIP (Phylogeny Inference Package) version 3.6. *Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.*

Finch MO, Lambert DM (1996) Kinship and genetic divergence among populations of tuatara *Sphenodon punctatus* as revealed by minisatellite DNA profiling. Mol Ecol 5:651-658

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conserv Biol 10:1500-1508

Fraser WM (1925) The Poor Knights Islands: A brief account of the Maori occupation. NZ J Sci Tech 8:8-14

Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. Mol Ecol 8:1513-1520

Gaze P (2001) Tuatara recovery plan 2001-2011. Threatened Species Recovery Plan Series No. 47. New Zealand Department of Conservation, Wellington

Goldstein DB, Ruiz Linares A, Cavalli-Sforza LL, Feldman MW (1995) Genetic absolute dating based on microsatellites and the origin of modern humans. Proc Natl Acad Sci USA 92:6723-6727

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available via http://www.unil.ch/izea/softwares/fstat.html Accessed Feb 2002

Goudet J, Raymond M, Demeeus T, Rousset F (1996) Testing differentiation in diploid populations. Genetics 144:1933-1940

Hanley TC, Caccone A (2005) Development of primers to characterize the mitochondrial control region of Galápagos land and marine iguanas (*Conolophus* and *Amblyrynchus*). Mol Ecol Notes 5:599-601

Hay JM, Daugherty CH, Cree A, Maxson LR (2003) Low genetic divergence obscures phylogeny among populations of *Sphenodon*, remnant of an ancient reptile lineage. Mol Phylogenet Evol 29:1-19

Hay JM, Sarre S, Daugherty CH (2004) Nuclear mitochondrial pseudogenes as molecular outgroups for phylogenetically isolated taxa: a case study in *Sphenodon*. Heredity 93:468-475

Hayward BW (1986) Origin of the offshore islands of northern New Zealand and their landform development. The Offshore Islands of Northern New Zealand, New Zealand Department of Lands and Survey Information Series 16:129-138

Hayward BW (1991) Geology and geomorphology of the Poor Knights Islands, northern New Zealand. Tane 33:23-37

Keogh JS, Scott IAW, Hayes C (2005) Rapid and repeated origin of insular gigantism and dwarfism in Australian tiger snakes. Evolution 59:226-233

King M (2003) The Penguin History of New Zealand. Penguin Books, Auckland

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics 5:150-163

Lande R (1999) Extinction risks from anthropogenic, ecological and genetic factors. In: Landweber LF, Dobson AP (eds) Genetics and the extinction of species: DNA and the conservation of biodiversity, Princeton University Press, Princeton, NJ

Leberg PL (1992) Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution 46:477-494

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998a) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered 89:238–247

Luikart G Cornuet JM (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conserv Biol 12:228-237

Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998b) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Mol Ecol 7:963–974

MacAvoy ES, McGibbon LM, Sainsbury JP et al. (2007) Genetic variation in island populations of tuatara (*Sphenodon* spp) inferred from microsatellite markers. Conserv Genet 8:305-318

Mayr E (1963) Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge

Menozzi P, Piazza A, Cavalli-Sforza L (1978) Synthetic maps of human gene frequencies in Europeans. Science 201:786-792

Moore JA, Nelson NJ, Keall SN, Daugherty CH (2008) Implications of social dominance and multiple paternity for the genetic diversity of a captive-bred reptile population (tuatara). Conserv Genet 9:1243-1252.

Moritz C (1994) Defining 'evolutionarily significant units' for conservation. Trends Ecol Evol 9:373-375

Moritz C (1999) Conservation units and translocations: strategies for conserving evolutionary processes. Hereditas 130:217-228

Nei M (1978) Estimation of average heterozygosity and genetic distance from a number of individuals. Genetics 89:538-590

Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. J Mol Evol 19:153-170

Nelson NJ, Keall SN, Brown D, Daugherty CH (2002a) Establishing a new wild population of tuatara (*Sphenodon guntheri*). Conserv Biol 16:887-894

Nelson NJ, Keall SN, Pledger S, Daugherty CH (2002b) Male-biased sex ratio in a small tuatara population. J Biogeogr 29:633-640

Newman DG (1986) Can tuatara and mice co-exist? The status of tuatara, *Sphenodon punctatus* (Reptilia: Rhynchocephalia), on the Whangamata Islands. The Offshore Islands of New Zealand, New Zealand Department of Lands and Survey Information Series 16:179-185

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 4:347-354

Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. Genetics 147:1943-1957

Parent CE, Caccone A, Petren K (2008) Colonization and diversification of Galápagos terrestrial fauna: a phylogenetic and biogeographical synthesis. Proc R Soc B 363:3347-3361

Paterson H (2005) The competitive Darwin. Paleobiology 31:56-76

Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502-503

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817-818

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248-249

Reich D, Price AL, Patterson N (2008) Principal component analysis of genetic data. Nat Genet 40:491-492

Rest JS, Ast JC, Austin CC et al. (2003) Molecular systematics of primary reptilian lineages and the tuatara mitochondrial genome. Mol Phylogenet Evol 29:289-297

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223-225

Ryder OA (1986) Species conservation and systematics: The dilemma of subspecies. Trends Ecol Evol 1:9-10

Sarre SD, Schwaner T, Georges A (1990) Genetic Variation among Insular Populations of the Sleepy Lizard, *Trachydosaurus rugosus* Gray (Squamata : Scincidae). Aust. J Zool. 38:603-616

Schneider S, Roessli D, Excoffier L (2000) Arlequin ver. 2.000: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland

Schwaner TD (1985) Population structure of black tiger snakes, *Notechis ater niger*, on offshore islands of south Australia. In: Grigg G, Shine R, Ehmann H (eds) Biology of Australasian Frogs and Reptiles. Royal Zoological Society of New South Wales, pp. 35–46

Soulé M, Yang SY (1973) Genetic Variation in Side-Blotched Lizards on Islands in the Gulf of California. Evolution 27:593-600

Subramanian S, Hay JM, Mohandesan E, Millar CD, Lambert DM (2008) Molecular and morphological evolution in tuatara are decoupled. Trends in Genetics 25:16-18

Swofford DL (2003) PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts

Terrasa B, Pérez-Mellado V, Brown RP, Picornell A, Castro JA, Ramon MM (2009) Foundations for conservation of intraspecific genetic diversity revealed by analysis of phylogeographical structure in the endangered endemic lizard *Podarcis lilfordi*. Diversity Distrib 15:207-221

Waples RS (1991) Pacific salmon, *Onchorhynchus* spp., and the definition of 'species' under the endangered species act. Mar Fish Review 53:11-22

Waser PM, Strobeck C (1998) Genetic signatures of interpopulation dispersal. Trends Ecol Evol 13:43-44

Whitaker AH (1968) The lizards of the Poor Knights Islands, New Zealand. NZ J Sci 11:623-651

Whittaker RJ, Triantis KA, Ladle RJ (2008) A general dynamic theory of oceanic island biogeography. J Biogeogr 35:977-944

Wilson AC, Cann RL, Carr SM et al. (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. Biol J Linn Soc Lond 26:375-400

ACKNOWLEDGEMENTS

We thank Niccy Aitken and members of the Sarre and Lambert labs for technical and analytical assistance. JMH and this research were funded by a New Zealand Foundation for Research Science

and Technology Postdoctoral Fellowship, and subsequently a Marsden Fund grant to DML and the Allan Wilson Centre for Molecular Ecology and Evolution.

Figure Legends

Figure 1. Distribution of natural populations of *Sphenodon* in New Zealand. Sampled populations are: ▼ northern *S. punctatus* (N): 1. Poor Knights Islands (Tawhiti Rahi, Aorangi, Aorangaia, Stack B), 2. Hen and Chickens Islands (Hen, Lady Alice, Whatupuke, Coppermine), 3. Little Barrier Island, 4. Cuvier Island, 5. Mercury Islands (Stanley, Red, Middle and Green), 6. Aldermen Islands, (Ruamahua-iti, Ruamahua-nui, Hongiora, Hernia), and the Bay of Plenty Islands, 7. Karewa Island, 8. Motunau Island, and 9. Moutoki Island, ▲ western Cook Strait *S. punctatus* (WCS): 10. Stephens Island, 11. Trios Islands (Middle, North, South), and ■ *S. guntheri* 12.North Brother Island (Bro). Regional names mentioned in text are in italics. Inset: summary of phylogenetic trees produced by previous studies of allozymes and mtDNA (Daugherty *et al.* 1990; Hay *et al.* 2003).

Figure 2. Expected heterozygosities of microsatellite DNA (H_M) and allozyme (H_A) compared to log of island size. R-squared regression lines shown.

Figure 3. Principal component analyses of, a) microsatellite data, and b) allozyme data.

Figure 4. Minimum evolution tree with Kimura-2 distances of 1039 bp mitochondrial control region and flanking tRNA sequence of 106 tuatara representing all sampled 26 populations. Colours indicate the 12 island groups (see Figure 1). Support for nodes on branches are confidence probabilities/bootstrap confidence levels x1000 replications, only values above 50% are shown. Root of tree is between northern and Cook Strait populations as identified in Hay *et al.* (2004).

Figure 5. Unrooted median–joining network of 1039 bp mitochondrial control region and flanking tRNA sequence of 106 tuatara, which reduce to 50 haplotypes. Colours indicate the 14 island groups (see map insert and Figure 1). Black nodes are hypothesised intermediary genetic haplotypes not found in our sample. Size of circle indicates number of individuals with that haplotype. Branch length indicates relative number of mutations between haplotypes.

Figure 6. Maximum parsimony 50% majority rule tree, with bootstrap confidence values >50% (x100 replications) of the 50 haplotypes found in the mtDNA.

Figure 7. Unrooted minimum evolution tree from Nei's Da distances of 5 microsatellite loci of 144 tuatara individuals. Colours indicate the 14 island groups (see Figure 1).

Table 1. Tuatara mitochondrial DNA mean between-population distances (Kimura-2), with mean within-population distances on the diagonal in italics, calculated with MEGA 3.1 with pair-wise deletion option. An asterisk indicates groups that contain additional variation at gap sites.

Island	n	TR	Aor	Aaia	StkB	Hen	LAl	Wht	Cop	LBI	Cuv	Red	Stan	MMc	Grn
TawhitiRahi	5	*0.004*													
Aorangi	5	0.003	*0.004*												
Aorangaia	1	0.002	0.004	*n/c*											
StackB	2	0.002	0.004	0	*0*										
Hen	4	0.008	0.008	0.007	0.007	*0.01*									
LadyAlice	4	0.005	0.006	0.002	0.002	0.008	*0.003*								
Whatupuke	2	0.004	0.006	0.002	0.002	0.008	0.002	*0.004*							
Coppermine	4	0.003	0.004	0	0	0.007	0.002	0.002	*0.001**						
LittleBarrier	4	0.006	0.007	0.005	0.005	0.008	0.005	0.005	0.005	*0**					
Cuvier	4	0.008	0.009	0.006	0.006	0.01	0.006	0.006	0.006	0.004	*0.002*				
RedMerc	2	0.007	0.008	0.006	0.006	0.01	0.006	0.006	0.006	0.005	0.004	*0*			
Stanley	3	0.006	0.007	0.004	0.004	0.009	0.005	0.005	0.004	0.003	0.004	0.004	*0.002*		
MiddelMerc	2	0.006	0.007	0.005	0.005	0.011	0.007	0.007	0.005	0.006	0.006	0.007	0.003	*0*	
Green	3	0.006	0.007	0.005	0.005	0.01	0.006	0.006	0.005	0.003	0.005	0.006	0.002	0.003	*0.003*
Rua-nui	6	0.009	0.009	0.008	0.008	0.009	0.008	0.008	0.008	0.008	0.009	0.01	0.008	0.011	0.009
Rua-iti	6	0.006	0.007	0.004	0.004	0.008	0.004	0.004	0.004	0.002	0.004	0.005	0.004	0.006	0.005
Hongiora	5	0.008	0.009	0.006	0.006	0.01	0.006	0.006	0.006	0.005	0.006	0.008	0.006	0.009	0.008
Hernia	3	0.008	0.009	0.006	0.006	0.01	0.006	0.006	0.006	0.005	0.006	0.008	0.006	0.009	0.008
Karewa	6	0.008	0.008	0.007	0.007	0.008	0.007	0.007	0.007	0.005	0.007	0.008	0.007	0.009	0.008
Motunau	6	0.011	0.011	0.011	0.011	0.007	0.011	0.011	0.011	0.012	0.013	0.013	0.013	0.016	0.014
Moutoki	6	0.012	0.012	0.012	0.012	0.013	0.012	0.012	0.012	0.011	0.014	0.014	0.014	0.017	0.014
Stephens	8	0.013	0.012	0.013	0.013	0.014	0.014	0.014	0.014	0.014	0.016	0.015	0.014	0.016	0.015
MiddleTrio	5	0.013	0.013	0.013	0.013	0.014	0.014	0.013	0.013	0.015	0.016	0.016	0.016	0.018	0.017
SouthTrio	1	0.013	0.013	0.013	0.013	0.014	0.014	0.013	0.013	0.015	0.016	0.016	0.016	0.018	0.017
NorthTrio	2	0.012	0.012	0.012	0.012	0.014	0.013	0.013	0.013	0.014	0.015	0.015	0.015	0.017	0.016
Brothers	7	0.016	0.015	0.016	0.016	0.017	0.017	0.017	0.017	0.016	0.017	0.017	0.017	0.019	0.018

Island	n	Rnui	Riti	Hon	Her	Kar	Mot	Mou	Stp	MTr	STr	NTr	Bro
Rua-nui	6	*0.009*											
Rua-iti	6	0.007	*0.002*										
Hongiora	5	0.009	0.003	*0*									
Hernia	3	0.009	0.003	0	*0*								
Karewa	6	0.008	0.005	0.008	0.008	*0.006*							
Motunau	6	0.007	0.011	0.013	0.013	0.008	*0**						
Moutoki	6	0.013	0.012	0.012	0.012	0.013	0.013	*0*					
Stephens	8	0.015	0.014	0.016	0.016	0.014	0.014	0.014	*0.003**				
MiddleTrio	5	0.014	0.014	0.015	0.015	0.014	0.013	0.014	0.003	*0**			
SouthTrio	1	0.014	0.014	0.015	0.015	0.014	0.013	0.014	0.003	0	*n/c*		
NorthTrio	2	0.014	0.014	0.014	0.014	0.014	0.012	0.013	0.003	0	0	*0.001*	
Brothers	7	0.017	0.017	0.018	0.018	0.016	0.016	0.017	0.004	0.003	0.003	0.004	*0*

Table 2. Microsatellite sample sizes (n), heterozygosities (H_M), and number of alleles (n_A) per locus per population or island group, and number of polymorphic loci per population or island group. Number of private alleles are in brackets.

Islands	Island Group	Pop	1/B3		1/A6		2/A12		1/C1		1/C2		All Loci		Number poly-
		n	H_M	n_A	H_M	n_A	H_M	n_A	H_M	n_A	H_M	n_A	Ave H_M	Tot n_A	morphic loci
Tawhiti Rahi	Poor Knights	6	0.68	6	0.17	2	0.56	4	0	1	0.17	2	0.32	15	4
Aorangi	Poor Knights	6	0.8	5	0.41	2	0.53	3	0	1	0.3	2	0.41	13	4
Aorangaia	Poor Knights	1	0	1	1	2	0	1	0	1	0	1	0.2	6	1
Stack B	Poor Knights	2	0.84	3	0	1	0	1	0	1	0	1	0.17	7	1
Ave/Total(T)	**Poor Knights**	**15T**	**0.58**	**6T(1)**	**0.40**	**2T**	**0.27**	**5T**	**0**	**1T**	**0.12**	**2T**	**0.28**	**16T(1)**	**4**
Hen	Hen & Chickens	6	0.76	4	0.66	3	0.85	6	0.91	7	0.32	3(1)	0.7	23(1)	5
Lady Alice	Hen & Chickens	6	0.82	6	0.44	3	0.88	7	0.66	5	0.68	3	0.7	24	5
Whatupuke	Hen & Chickens	6	0.64	4	0.44	3	0.83	5	0.79	5	0.55	2	0.65	19	5
Coppermine	Hen & Chickens	6	0.8	5	0.49	2	0.86	6(1)	0.86	5	0.44	3	0.69	21(1)	5
Ave/Total(T)	**Hen & Chickens**	**24T**	**0.76**	**12T(1**	**0.51**	**5T**	**0.86**	**15T**	**0.81**	**8T**	**0.50**	**4T**	**0.69**	**44T(1)**	**5**
Little Barrier	Little Barrier	6	0.89	7	0.74	4(1)	0.68	5	0.65	4	0.44	3	0.68	23(1)	5
Cuvier	Cuvier	4	0.64	4	0.61	3(1)	0.68	3	0.43	2	0.54	2	0.58	14(1)	5
Red Mercury	Mercuries	3	0.6	3(1)	0.53	2	0.87	4	0.8	4	0.53	2	0.67	15(1)	5
Stanley	Mercuries	6	0.86	6	0.55	3	0.93	8	0.88	6	0.79	5	0.8	28	5
Middle Mercury	Mercuries	6	0.86	6	0.49	2	0.68	4	0.55	2	0.17	2	0.55	16	5
Green Mercury	Mercuries	6	0.76	4	0.55	2	0.62	3	0.55	2	0.3	2	0.56	13	5
Ave/Total(T)	**Mercuries**	**21T**	**0.77**	**14T**	**0.53**	**3T**	**0.78**	**15T**	**0.70**	**8T**	**0.45**	**5T**	**0.65**	**45T**	**5**
Ruamahua-nui	Aldermen	6	0.85	6	0	1	0.85	6	0.79	4(1)	0.73	4	0.64	21(1)	4
Ruamahua-iti	Aldermen	6	0.79	5	0.3	2	0.68	6(1)	0.8	5	0.44	3	0.6	21(1)	5
Hongiora	Aldermen	5	0.84	5	0.71	3	0.8	4	0.6	3	0.73	4	0.74	19	5
Hernia	Aldermen	3	0.6	3	0.53	2	0.33	2	0.53	2	0.87	4	0.57	13	5
Ave/Total(T)	**Aldermen**	**20T**	**0.77**	**10T**	**0.39**	**3T**	**0.67**	**13T**	**0.68**	**7T**	**0.69**	**6T**	**0.64**	**39T**	**5**
Karewa	Karewa	6	0.76	4	0.41	2	0.73	5(1)	0.82	5	0	1	0.54	17(1)	4
Motunau	Motunau	6	0.71	3	0	1	0.59	3	0.74	4	0.44	3	0.5	14	4
Moutoki	Rurimas	6	0	1	0.55	2	0.55	2(2)	0	1	0.48	2	0.32	8(2)	3
Stephens	Stephens	7	0.91	8	0.54	3	0.86	7(1)	0.48	3	0.49	2	0.66	23(1)	5
Middle Trio	Trios	6	0.74	5	0.17	2	0.76	5	0.71	4	0.17	2	0.51	18	5
South Trio	Trios	2	0.65	2	0.5	2	0	1	0.5	2	0	1	0.33	8	3
North Trio	Trios	3	0	1	0	1	0.6	2	0.33	2	0	1	0.19	7	2
Ave/Total(T)	**Trios**	**11T**	**0.46**	**7T**	**0.22**	**3T**	**0.45**	**6T**	**0.51**	**5T**	**0.06**	**2T**	**0.34**	**23T**	**5**
North Brother	Brothers	18	0.2	2	0	1	0.46	2	0	2	0	1	0.13	8	3
Average			0.65	4.1	0.42	2.2	0.62	4	0.51	3.2	0.37	2.3	0.51	15.9	4.35
Total all samples		144		21		8		26		9		8			

Table 3. Allozyme sample sizes (n), heterozygosities (H_A) per and number of polymorphic loci per population or island group, and number of alleles (n_A) per locus per population or island group. Tuatara island sizes and population size estimates. Tuatara current population size estimate from Gaze (2001): "100s" = 500 here, "few 100s" = 300, "high 100s-low 1000s" = 1000 etc. North Brother population size is from Nelson *et al.* 2002b.

Islands	Island Group	Pop	Gp-2	Gp-6	Gus-1	Hb-2	Icd-1	Mdh-1	Mpi-1	Pgm-1	Pgm-2	Tot	Number poly-	All Loci	Isl size	Size pop
		n	n_A	n_A	n_A	n_A	n_A	n_A	n_A	n_A	n_A	n_A	morphic loci	Ave H_A	(ha)	
Tawhiti Rahi	Poor Knights	15	1	1	1	1	1	2	1	1	1	10	1	0.02	163	1000
Aorangi	Poor Knights	20	1	1	1	1	1	2	1	1	1	10	1	0.021	110	1000
Aorangaia	Poor Knights	1	1	1	1	1	1	2	1	1	1	10	1	0.043	6.3	30
Stack B	Poor Knights	3	1	1	1	1	1	2	1	1	1	10	1	0.026	0.8	30
Ave/Total(T)	**Poor Knights**	**39T**	**1T**	**1T**	**1T**	**1T**	**1T**	**2T**	**1T**	**1T**	**1T**	**10T**	**1T**	**0.028**	**280.1T**	**2060T**
Hen	Hen & Chickens	15	1	1	1	1	2	2	1	1	1	11	2	0.009	500	300
Lady Alice	Hen & Chickens	15	1	1	1	2	1	2	1	2	1	12	3	0.021	155	1000
Whatupuke	Hen & Chickens	15	1	1	1	2	2	2	1	3	1	14	4	0.032	101.9	300
Coppermine	Hen & Chickens	15	1	1	1	1	1	2	1	2	1	11	2	0.025	79.5	300
Ave/Total(T)	**Hen & Chickens**	**60T**	**1T**	**1T**	**1T**	**3T**	**2T**	**2T**	**1T**	**3T**	**1T**	**15T**	**4T**	**0.022**	**836.4T**	**1900T**
Little Barrier	Little Barrier	4	1	1	1	1	1	2	1	1	1	10	1	0.011	3083	30
Cuvier	Cuvier	4	1	1	1	2	2	1	1	2	1	12	3	0.045	170	20
Red Mercury	Mercuries	11	1	1	1	2	2	2	1	2	1	13	4	0.05	225	30
Stanley	Mercuries	13	1	2	1	2	1	2	1	1	1	12	3	0.04	99.5	30
Middle Mercury	Mercuries	17	1	2	1	2	1	2	1	2	1	13	4	0.032	13.1	3000
Green Mercury	Mercuries	15	1	1	1	2	1	1	1	1	2	11	2	0.019	2.3	500
Ave/Total(T)	**Mercuries**	**56T**	**1T**	**2T**	**1T**	**2T**	**2T**	**2T**	**1T**	**2T**	**2T**	**15T**	**6T**	**0.035**	**339.9T**	**3560T**
Ruamahua-nui	Aldermen	7	1	1	1	2	1	2	1	1	2	12	3	0.034	32.5	500
Ruamahua-iti	Aldermen	16	1	1	1	2	1	2	2	1	2	13	4	0.055	25	500
Hongiora	Aldermen	5	1	1	1	1	1	2	1	1	1	10	1	0.023	16.3	300
Hernia	Aldermen	3	1	1	1	3	1	1	1	1	1	11	1	0.026	3.4	100
Ave/Total(T)	**Aldermen**	**31T**	**1T**	**1T**	**1T**	**3T**	**1T**	**2T**	**2T**	**1T**	**2T**	**14T**	**4T**	**0.035**	**77.2T**	**1400T**
Karewa	Karewa	15	1	1	1	2	1	1	1	1	1	10	1	0.003	5	500
Motunau	Motunau	15	1	1	1	2	1	1	1	1	1	10	1	0.02	3.6	1000
Moutoki	Rurimas	15	2	1	1	2	1	1	1	2	1	12	3	0.017	0.8	200
Stephens	Stephens	15	1	2	1	1	2	2	1	1	1	12	3	0.033	150	30000
Middle Trio	Trios	19	1	1	1	1	1	2	1	1	1	10	1	0.013	20	3000
South Trio	Trios	3	1	1	1	1	1	1	1	1	1	9	0	0	2	30
North Trio	Trios	3	1	1	1	1	1	1	1	1	1	9	0	0	1	30
Ave/Total(T)	**Trios**	**25T**	**1T**	**1T**	**1T**	**1T**	**1T**	**2T**	**1T**	**1T**	**1T**	**10**	**1T**	**0.004**	**23T**	**3060T**
North Brother	Brothers	13	2	2	1	1	1	1	1	1	1	11	2	0.013	4	450
Average														0.024	191	1573
Total all samples		293	2	3	2	3	2	2	2	3	2	21	9		4973	44180

Table 4. Summary table of correct and wrong assignments of individual tuatara to source population from microsatellite data (from Arlequin). Rows = source population. Shaded populations are those with all individuals correctly assigned, and to which no individuals from other populations were incorrectly assigned.

Island	Island Group (see Fig 1)	Number individuals correctly assigned	Number individuals incorrectly assigned	Population to which false assignment made
Tawhiti_Rahi	Poor Knights	4	2	Aorangi, PK
Aorangi	Poor Knights	6	-	-
Aorangaia	Poor Knights	0	1	Tawhiti Rahi, PK
Stack_B	Poor Knights	2	-	-
Hen	Hen and Chickens	6	-	-
Lady_Alice	Hen and Chickens	5	1	Whatupuke, HnC
Whatupuke	Hen and Chickens	5	1	Motunau, Mot
Coppermine	Hen and Chickens	5	1	Whatupuke, HnC
Little_Barrier	Little Barrier	6	-	-
Cuvier	Cuvier	3	1	Lady Alice, HnC
Red_Mercury	Mercuries	3	-	-
Stanley	Mercuries	6	-	-
Middle_Mercury	Mercuries	6	-	-
Green_Mercury	Mercuries	6	-	-
Ruamahua-nui	Aldermen	6	-	-
Ruamahua-iti	Aldermen	6	-	-
Hongiora	Aldermen	5	-	-
Hernia	Aldermen	3	-	-
Karewa	Karewa	6	-	-
Motunau	Motunau	6	-	-
Moutoki	Rurimas	6	-	-
Stephens	Stephens	7	-	-
Middle_Trio	Trios	6	-	-
South_Trio	Trios	2	-	-
North_Trio	Trios	3	-	-
Brothers	Brothers	7*	-	-

7* Brothers has 7 unique microsatellite genotypes in 18 individuals

Table 5. Summary table of correct and wrong assignments of individual tuatara to source island group from microsatellite data (from Arlequin). Rows = source island group. Shaded island groups are those with all individuals correctly assigned, and to which no individuals from other island groups were incorrectly assigned.

Island Group (see Fig. 1)	Number of individuals correctly assigned	Number of individuals incorrectly assigned	Island group to which false assignment made
PoorKnights	13	0	-
Hen and Chickens	23	1	Motunau
Little Barrier	6	0	-
Cuvier	4	0	-
Mercuries	17	4	Aldermen, Little Barrier(2)
Aldermen	20	0	-
Karewa	6	0	-
Motunau	6	0	-
Moutoki	6	0	-
Stephens	7	0	-
Trios	11	0	-
Brothers	7	0	-

7* Brothers has 7 unique microsatellite genotypes in 18 individuals

Table 6. AMOVA analysis of contributions of genetic variation to population structure in microsatellites, allozymes and mtDNA (Arlequin). "3 groups" of populations are the allozyme defined groups of North Brother, Western Cook Strait and northern populations. "2 groups" of populations are the mitochondrial/geographic defined groups of Cook Strait and northern populations. * $p < 0.05$, ** $p < 0.01$.

Source of variation	Genetic structure	Data	Percentage of variation	Significance
Among groups	3 groups	microsat	8.06	*
	2 groups	microsat	4.64	*
	3 groups	allozyme	67.18	**
	2 groups	allozyme	52.61	**
	3 groups	mtDNA	55.7	**
	2 groups	mtDNA	57.2	**
Among populations within groups	3 groups	microsat	27.89	**
	2 groups	microsat	30.1	**
	3 groups	allozyme	8.42	**
	2 groups	allozyme	18.95	**
	3 groups	mtDNA	27.9	**
	2 groups	mtDNA	26.94	**
Within populations	3 groups	microsat	64.05	**
	2 groups	microsat	65.26	**
	3 groups	allozyme	24.4	**
	2 groups	allozyme	28.44	**
	3 groups	mtDNA	16.4	**
	2 groups s	mtDNA	15.86	**

Figure 1.

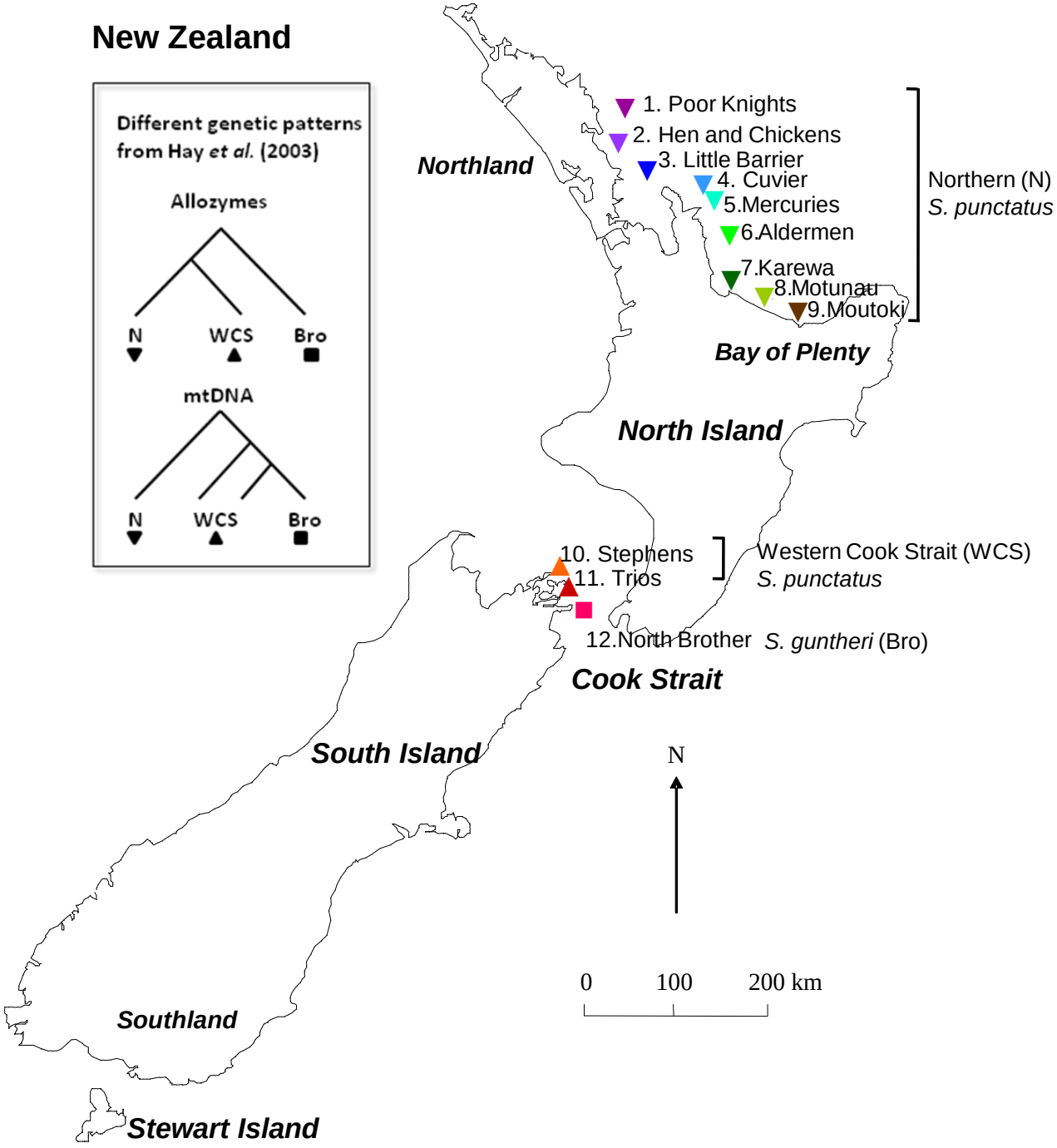
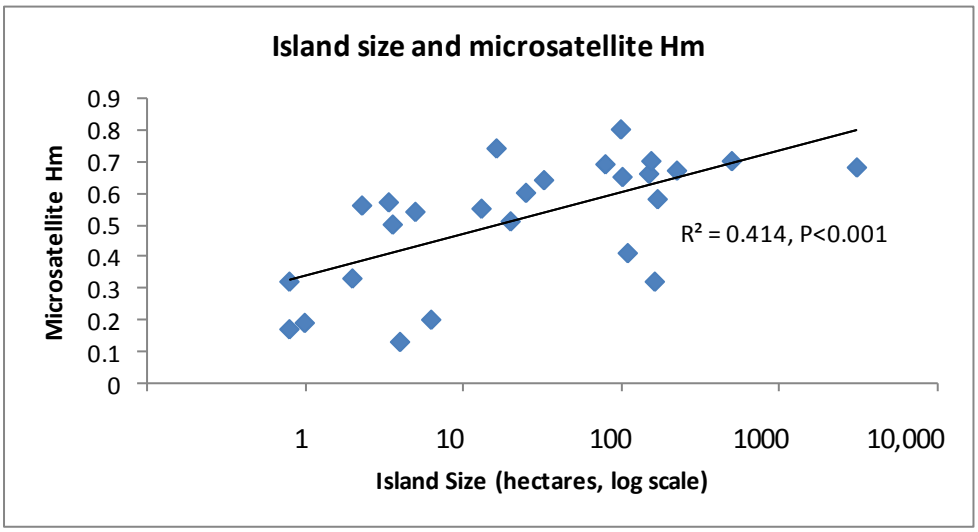

New Zealand
Different genetic patterns from Hay et al. (2003)
Allozymes
N
WCS
Bro
mtDNA
N
WCS
Bro
Northland
1. Poor Knights
2. Hen and Chickens
3. Little Barrier
4. Cuvier
5.Mercuries
6.Aldermen
7.Karewa
8.Motunau
9.Moutoki
Northern (N)
S. punctatus
Bay of Plenty
North Island
10. Stephens
11. Trios
Western Cook Strait (WCS)
S. punctatus
12.North Brother
S. guntheri (Bro)
Cook Strait
South Island
N
0
100
200 km
Southland
Stewart Island

Figure 2. Expected heterozygosities of microsatellite DNA (H_M) and allozyme (H_A) compared to log of island size. R-squared regression lines shown.



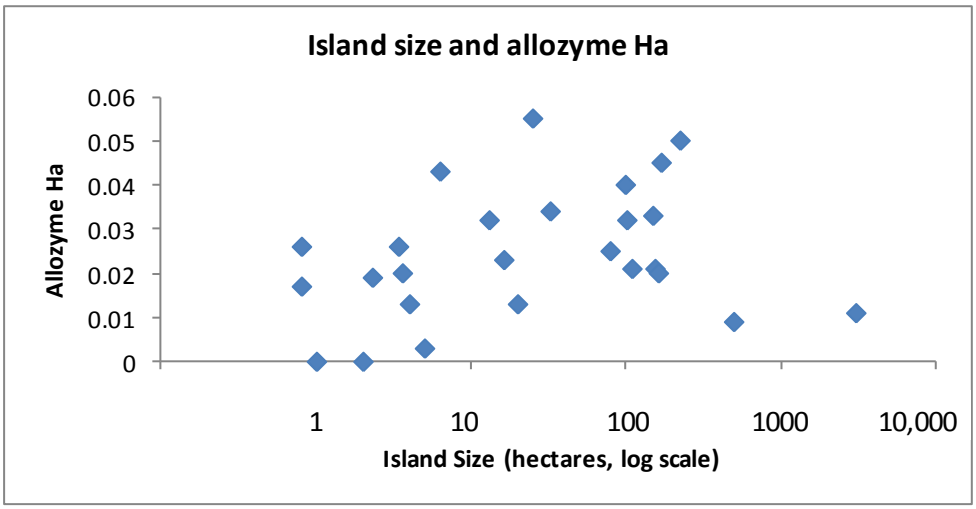


Figure 3a. Principal component analysis of microsatellite data

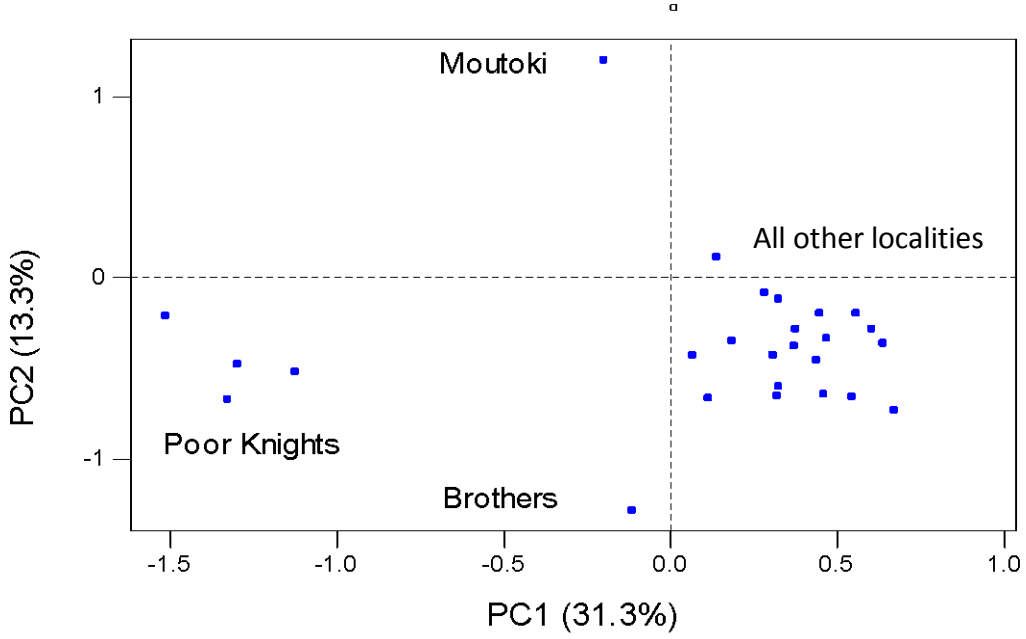


Figure 3b. Principal component analysis of allozyme data

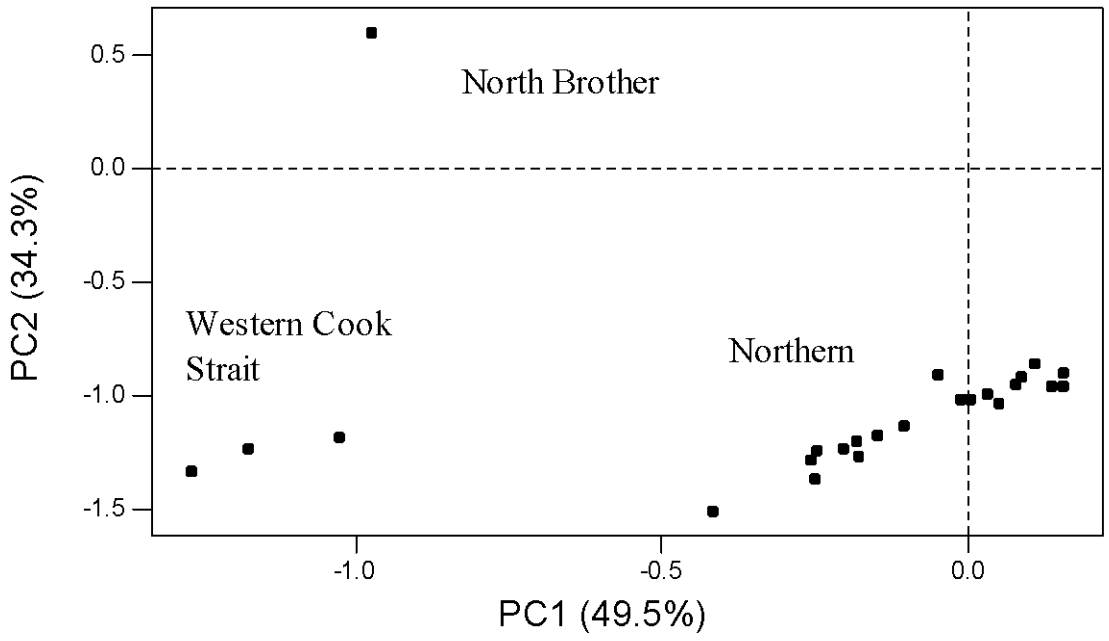


Figure 4.

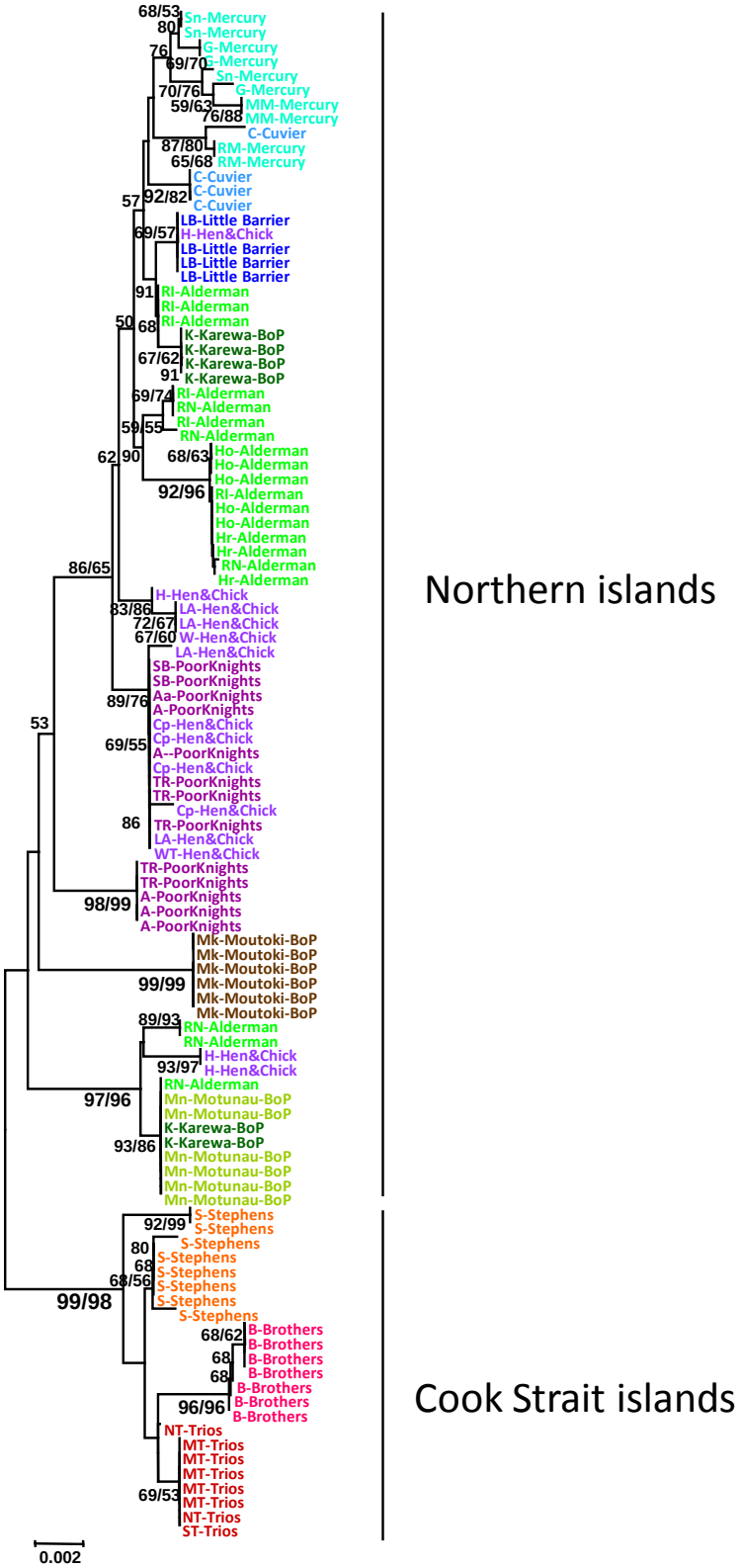

Sn-Mercury
Sn-Mercury
G-Mercury
G-Mercury
Sn-Mercury
G-Mercury
MM-Mercury
MM-Mercury
C-Cuvier
RM-Mercury
RM-Mercury
C-Cuvier
C-Cuvier
C-Cuvier
LB-Little Barrier
H-Hen&Chick
LB-Little Barrier
LB-Little Barrier
LB-Little Barrier
RI-Alderman
RI-Alderman
RI-Alderman
K-Karewa-BoP
K-Karewa-BoP
K-Karewa-BoP
K-Karewa-BoP
RI-Alderman
RN-Alderman
RI-Alderman
RN-Alderman
Ho-Alderman
Ho-Alderman
Ho-Alderman
RI-Alderman
Ho-Alderman
Ho-Alderman
Hr-Alderman
Hr-Alderman
RN-Alderman
Hr-Alderman
H-Hen&Chick
LA-Hen&Chick
LA-Hen&Chick
W-Hen&Chick
LA-Hen&Chick
SB-PoorKnights
SB-PoorKnights
Aa-PoorKnights
A-PoorKnights
Cp-Hen&Chick
Cp-Hen&Chick
A--PoorKnights
Cp-Hen&Chick
TR-PoorKnights
TR-PoorKnights
Cp-Hen&Chick
TR-PoorKnights
LA-Hen&Chick
WT-Hen&Chick
TR-PoorKnights
TR-PoorKnights
A-PoorKnights
A-PoorKnights
A-PoorKnights
Mk-Moutoki-BoP
Mk-Moutoki-BoP
Mk-Moutoki-BoP
Mk-Moutoki-BoP
Mk-Moutoki-BoP
Mk-Moutoki-BoP
RN-Alderman
RN-Alderman
H-Hen&Chick
H-Hen&Chick
RN-Alderman
Mn-Motunau-BoP
Mn-Motunau-BoP
K-Karewa-BoP
K-Karewa-BoP
Mn-Motunau-BoP
Mn-Motunau-BoP
Mn-Motunau-BoP
Mn-Motunau-BoP
S-Stephens
S-Stephens
S-Stephens
S-Stephens
S-Stephens
S-Stephens
S-Stephens
B-Brothers
B-Brothers
B-Brothers
B-Brothers
B-Brothers
B-Brothers
B-Brothers
NT-Trios
MT-Trios
MT-Trios
MT-Trios
MT-Trios
MT-Trios
NT-Trios
ST-Trios
0.002
Northern islands
Cook Strait islands

Figure 5.

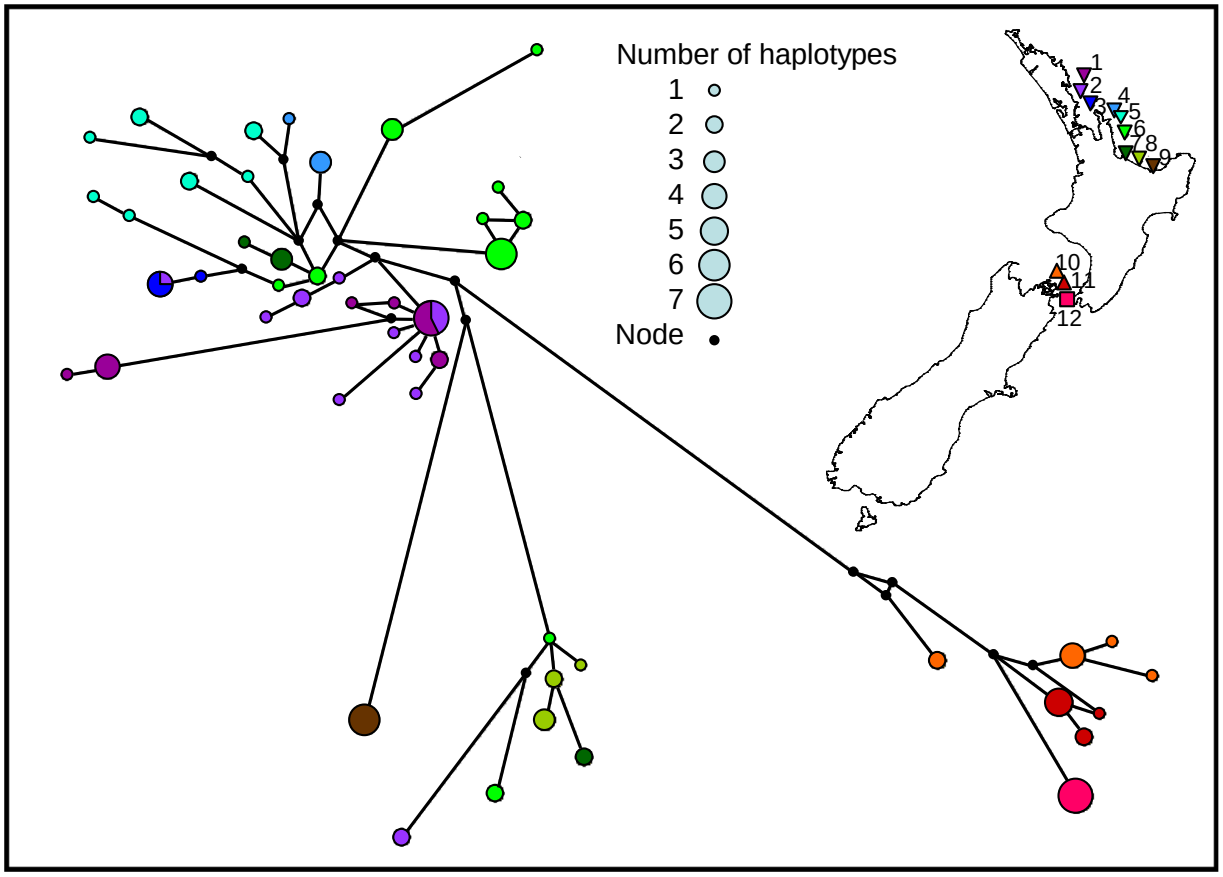


Figure 6.

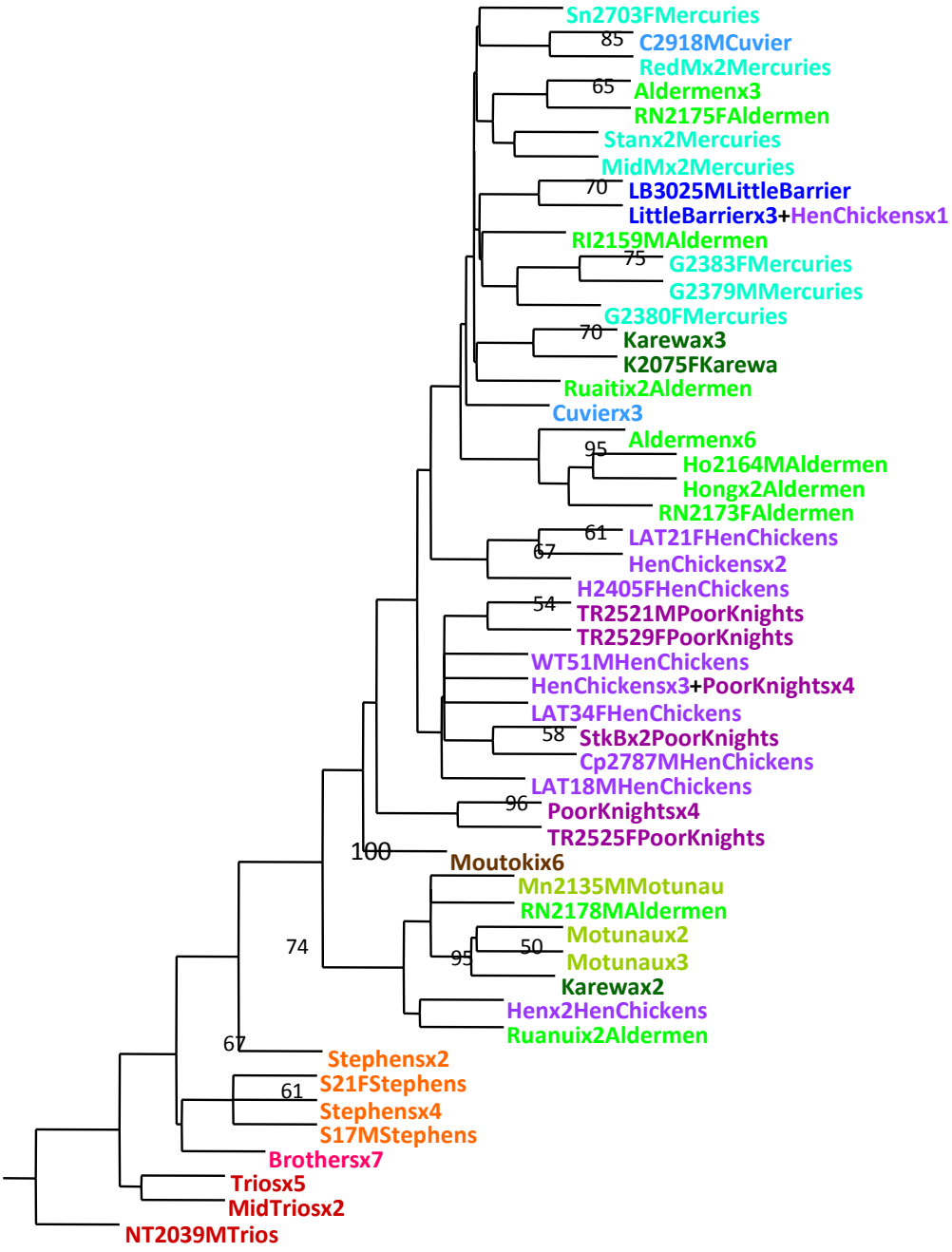
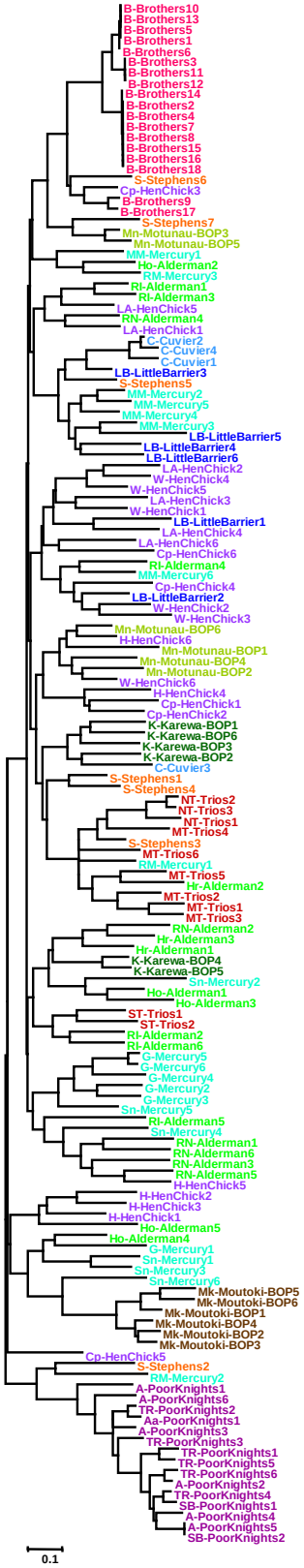

Sn2703FMercuries
85
C2918MCuvier
RedMx2Mercuries
65
Aldermenx3
RN2175FAldermen
Stanx2Mercuries
MidMx2Mercuries
70
LB3025MLittleBarrier
LittleBarrierx3+HenChickensx1
RI2159MAldermen
75
G2383FMercuries
G2379MMercuries
G2380FMercuries
70
Karewax3
K2075FKarewa
Ruaitix2Aldermen
Cuvierx3
Aldermenx6
95
Ho2164MAldermen
Hongx2Aldermen
RN2173FAldermen
61
LAT21FHenChickens
67
HenChickensx2
H2405FHenChickens
54
TR2521MPoorKnights
TR2529FPoorKnights
WT51MHenChickens
HenChickensx3+PoorKnightsx4
LAT34FHenChickens
58
StkBx2PoorKnights
Cp2787MHenChickens
LAT18MHenChickens
96
PoorKnightsx4
TR2525FPoorKnights
100
Moutokix6
Mn2135MMotunau
RN2178MAldermen
74
Motunaux2
50
95
Motunaux3
Karewax2
Henx2HenChickens
Ruanuix2Aldermen
67
Stephensx2
61
S21FStephens
Stephensx4
S17MStephens
Brothersx7
Triosx5
MidTriosx2
NT2039MTrios

Figure 7.


B-Brothers10
B-Brothers13
B-Brothers5
B-Brothers1
B-Brothers6
B-Brothers3
B-Brothers11
B-Brothers12
B-Brothers14
B-Brothers2
B-Brothers4
B-Brothers7
B-Brothers8
B-Brothers15
B-Brothers16
B-Brothers18
S-Stephens6
Cp-HenChick3
B-Brothers9
B-Brothers17
S-Stephens7
Mn-Motunau-BOP3
Mn-Motunau-BOP5
MM-Mercury1
Ho-Alderman2
RM-Mercury3
RI-Alderman1
RI-Alderman3
LA-HenChick5
RN-Alderman4
LA-HenChick1
C-Cuvier2
C-Cuvier4
C-Cuvier1
LB-LittleBarrier3
S-Stephens5
MM-Mercury2
MM-Mercury5
MM-Mercury4
MM-Mercury3
LB-LittleBarrier5
LB-LittleBarrier4
LB-LittleBarrier6
LA-HenChick2
W-HenChick4
W-HenChick5
LA-HenChick3
W-HenChick1
LB-LittleBarrier1
LA-HenChick4
LA-HenChick6
Cp-HenChick6
RI-Alderman4
MM-Mercury6
Cp-HenChick4
LB-LittleBarrier2
W-HenChick2
W-HenChick3
Mn-Motunau-BOP6
H-HenChick6
Mn-Motunau-BOP1
Mn-Motunau-BOP4
Mn-Motunau-BOP2
W-HenChick6
H-HenChick4
Cp-HenChick1
Cp-HenChick2
K-Karewa-BOP1
K-Karewa-BOP6
K-Karewa-BOP3
K-Karewa-BOP2
C-Cuvier3
S-Stephens1
S-Stephens4
NT-Trios2
NT-Trios3
NT-Trios1
MT-Trios4
S-Stephens3
MT-Trios6
RM-Mercury1
MT-Trios5
Hr-Alderman2
MT-Trios2
MT-Trios1
MT-Trios3
RN-Alderman2
Hr-Alderman3
Hr-Alderman1
K-Karewa-BOP4
K-Karewa-BOP5
Sn-Mercury2
Ho-Alderman1
Ho-Alderman3
ST-Trios1
ST-Trios2
RI-Alderman2
RI-Alderman6
G-Mercury5
G-Mercury6
G-Mercury4
G-Mercury2
G-Mercury3
Sn-Mercury5
RI-Alderman5
Sn-Mercury4
RN-Alderman1
RN-Alderman6
RN-Alderman3
RN-Alderman5
H-HenChick5
H-HenChick2
H-HenChick3
H-HenChick1
Ho-Alderman5
Ho-Alderman4
G-Mercury1
Sn-Mercury1
Sn-Mercury3
Sn-Mercury6
Mk-Moutoki-BOP5
Mk-Moutoki-BOP6
Mk-Moutoki-BOP1
Mk-Moutoki-BOP4
Mk-Moutoki-BOP2
Mk-Moutoki-BOP3
Cp-HenChick5
S-Stephens2
RM-Mercury2
A-PoorKnights1
A-PoorKnights6
TR-PoorKnights2
Aa-PoorKnights1
A-PoorKnights3
TR-PoorKnights3
TR-PoorKnights1
TR-PoorKnights5
TR-PoorKnights6
A-PoorKnights2
TR-PoorKnights4
SB-PoorKnights1
A-PoorKnights4
A-PoorKnights5
SB-PoorKnights2
0.1

Supplementary Material for Hay, Sarre, Lambert, Allendorf and Daugherty, Genetic diversity and taxonomy: a reassessment of species designation in tuatara (*Sphenodon*: Reptilia)

Population allele frequencies for five tuatara microsatellite DNA loci. Allele name is actual allele size

Locus 1/A6

Allele	190	192	194	196	198	200	204	206	2n
Brothers	0	0	0	0	0	0	1	0	36
Stephens	0.29	0	0	0	0	0.07	0.64	0	14
Middle Trio	0.92	0	0	0	0	0	0.08	0	12
North Trio	1	0	0	0	0	0	0	0	6
South Trio	0	0	0	0	0	0	0.75	0.25	4
Motunau	0	0	0	0	0	0	1	0	12
Moutoki	0	0	0	0	0	0	0.5	0.5	12
Karewa	0.25	0	0	0	0	0	0.75	0	12
Ruamahua-iti	0.17	0	0	0	0	0	0.83	0	12
Ruamahua-nui	0	0	0	0	0	0	1	0	12
Hongiora	0.4	0	0	0	0	0	0.4	0.2	10
Hernia	0.33	0	0	0	0	0	0.67	0	6
Middle Mercury	0.33	0	0	0	0	0	0.67	0	12
Green Mercury	0.5	0	0	0	0	0	0.5	0	12
Stanley	0.17	0	0	0	0	0	0.67	0.17	12
Red Mercury	0.33	0	0	0	0	0	0.67	0	6
Cuvier	0.13	0	0.25	0	0	0	0.63	0	8
Little Barrier	0.17	0.17	0	0	0.08	0	0.58	0	12
Hen	0	0	0	0.17	0	0	0.5	0.33	12
Lady Alice	0	0	0	0.08	0	0.17	0.75	0	12
Whatupuke	0.17	0	0	0.08	0	0	0.75	0	12
Coppermine	0	0	0	0.33	0	0	0.67	0	12
Tawhiti Rahi	0	0	0	0	0	0	0.08	0.92	12
Aorangi	0	0	0	0	0	0	0.25	0.75	12
Stack B	0	0	0	0	0	0	0	1	4
Aorangaia	0	0	0	0	0	0	0.5	0.5	2

Locus 1/B3

Allele	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	206	208	210	212	214	216	2n
Brothers	0	0	0	0	0	0	0	0	0	0.11	0	0	0	0.89	0	0	0	0	0	0	0	36
Stephens	0	0	0	0.07	0	0.07	0	0.14	0	0.21	0.21	0.07	0	0	0.14	0	0	0.07	0	0	0	14
Middle Trio	0	0	0	0	0	0	0	0	0	0.08	0	0	0.17	0.17	0.17	0	0	0	0	0.42	0	12
North Trio	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	6
South Trio	0	0	0	0	0	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	4
Motunau	0	0	0	0	0.25	0.42	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	12
Moutoki	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	12
Karewa	0	0.42	0	0	0.25	0	0	0	0	0.08	0.25	0	0	0	0	0	0	0	0	0	0	12
Ruamahua-iti	0	0	0	0.08	0	0.25	0	0	0.42	0.08	0.17	0	0	0	0	0	0	0	0	0	0	12
Ruamahua-nui	0	0.08	0.17	0	0	0	0	0	0.08	0	0.25	0.08	0	0.33	0	0	0	0	0	0	0	12
Hongiora	0	0	0	0	0	0	0	0.1	0.3	0.3	0.1	0.2	0	0	0	0	0	0	0	0	0	10
Hernia	0	0	0	0	0	0	0	0.17	0	0.17	0	0	0	0.67	0	0	0	0	0	0	0	6
Middle Mercury	0	0	0	0	0	0.17	0	0	0	0.08	0	0.33	0.08	0	0	0.17	0.17	0	0	0	0	12
Green Mercury	0	0	0	0	0	0	0	0	0.25	0	0.25	0	0.42	0	0	0.08	0	0	0	0	0	12
Stanley	0	0.08	0	0	0.17	0	0	0.08	0.17	0	0.17	0	0.33	0	0	0	0	0	0	0	0	12
Red Mercury	0	0	0	0	0	0	0.67	0	0	0	0	0	0	0.17	0	0	0	0	0	0.17	0	6
Cuvier	0	0	0	0	0	0	0	0.13	0	0	0	0.63	0.13	0	0	0	0.13	0	0	0	0	8
Little Barrier	0.08	0	0.08	0	0	0	0	0	0	0	0.25	0.25	0	0	0.08	0	0.08	0.17	0	0	0	12
Hen	0	0	0	0	0.25	0	0	0	0.08	0	0.42	0	0.25	0	0	0	0	0	0	0	0	12
Lady Alice	0	0.17	0	0	0	0	0	0	0	0.17	0.42	0	0	0	0.08	0	0.08	0	0	0	0.08	12
Whatupuke	0.08	0	0	0	0	0	0	0	0	0.25	0.58	0	0	0	0	0	0	0.08	0	0	0	12
Coppermine	0	0	0	0	0.17	0	0	0.08	0	0.33	0.33	0	0	0	0	0	0	0	0	0	0.08	12
Tawhiti Rahi	0	0	0	0	0	0	0	0	0	0	0.08	0.08	0	0.58	0.08	0.08	0	0	0.08	0	0	12
Aorangi	0	0	0	0	0	0	0	0	0	0	0.08	0	0	0.33	0.33	0.17	0	0	0.08	0	0	12
Stack B	0	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0.25	0.5	0	0	0	0	0	4
Aorangaia	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2

Locus 2/A12

Allele	152	154	156	158	160	162	164	166	168	170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	2n
Brothers	0	0	0	0.3	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	36
Stephens	0	0	0.1	0.3	0.1	0	0	0.1	0	0.3	0.1	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	14
Middle Trio	0	0	0	0	0.1	0	0.2	0.1	0	0.6	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
North Trio	0	0	0	0	0	0.5	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
South Trio	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	4
Motunau	0	0	0	0	0	0	0	0.6	0	0.3	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
Moutoki	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0.5	12
Karewa	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0.1	0	0	0.3	0	0	0	0.1	0	0	0	12
Ruamahua-iti	0	0	0	0.1	0	0	0	0	0.2	0.3	0	0	0	0	0	0	0.1	0	0	0.3	0	0.1	0	0	0	0	12
Ruamahua-nui	0	0	0	0	0.1	0	0	0	0	0	0.1	0	0	0.3	0	0	0.1	0.3	0.2	0	0	0	0	0	0	0	12
Hongiora	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0.1	0.3	0	0	0	0	0	0	0	10
Hernia	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0.8	0	0	0	0	0	0	0	6
Middle Mercury	0	0	0	0	0	0	0	0	0.1	0	0.5	0	0	0.1	0	0.3	0	0	0	0	0	0	0	0	0	0	12
Green Mercury	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0.1	0	0	12
Stanley	0	0	0	0.1	0	0.1	0.1	0	0	0	0.3	0.2	0	0	0	0	0.2	0.1	0	0.1	0	0	0	0	0	0	12
Red Mercury	0	0	0	0	0	0	0.2	0	0	0.3	0	0	0	0	0.2	0.3	0	0	0	0	0	0	0	0	0	0	6
Cuvier	0	0	0	0.4	0	0	0	0	0	0	0.5	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	8
Little Barrier	0	0	0	0	0.1	0	0	0.1	0	0.2	0.6	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	12
Hen	0	0	0	0	0	0	0.1	0.1	0	0.3	0	0	0.1	0.2	0	0	0	0.3	0	0	0	0	0	0	0	0	12
Lady Alice	0	0	0	0	0	0	0.1	0	0	0	0.3	0.1	0	0.1	0	0	0	0.1	0	0.2	0	0	0	0.2	0	0	12
Whatupuke	0	0	0	0	0	0	0.3	0	0.2	0.3	0.1	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	12
Coppermine	0.3	0	0	0	0	0	0	0	0	0	0	0.3	0.1	0	0	0	0.3	0	0.1	0	0.1	0	0	0	0	0	12
Tawhiti Rahi	0	0	0	0	0	0	0	0	0	0.1	0	0.7	0	0	0.1	0	0	0	0.2	0	0	0	0	0	0	0	12
Aorangi	0	0	0	0	0	0	0	0	0	0.1	0	0.7	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	12
Stack B	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	4
Aorangaia	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	2

Locus 1/C1

Allele	184	186	188	190	192	194	196	200	206	2n

Allele	184	186	188	190	192	194	196	200	206	Null	2n
Brothers	0	0	0.89	0	0	0	0	0	0	0.11	32
Stephens	0	0	0.71	0.14	0.14	0	0	0	0	0	14
Middle Trio	0.25	0	0.25	0.42	0.08	0	0	0	0	0	12
North Trio	0.17	0	0	0.83	0	0	0	0	0	0	6
South Trio	0	0	0	0.25	0	0.75	0	0	0	0	4
Motunau	0	0	0.42	0.33	0.08	0	0	0.17	0	0	12
Moutoki	0	0	0	0	1	0	0	0	0	0	12
Karewa	0.08	0.25	0	0.08	0.25	0.33	0	0	0	0	12
Ruamahua-iti	0.17	0	0	0.08	0.17	0.42	0	0.17	0	0	12
Ruamahua-nui	0	0	0	0.33	0	0.17	0	0.33	0.17	0	12
Hongiora	0.1	0	0	0	0	0.6	0.3	0	0	0	10
Hernia	0.67	0	0	0	0	0.33	0	0	0	0	6
Middle Mercury	0.5	0	0.5	0	0	0	0	0	0	0	12
Green Mercury	0	0	0	0	0	0	0.5	0.5	0	0	12
Stanley	0	0.17	0.08	0	0.25	0.25	0.08	0.17	0	0	12
Red Mercury	0	0	0	0.17	0.17	0.5	0	0.17	0	0	6
Cuvier	0	0.25	0	0	0	0.75	0	0	0	0	8
Little Barrier	0.58	0	0.17	0.17	0	0.08	0	0	0	0	12
Hen	0	0.08	0.08	0.17	0.17	0.08	0.25	0.17	0	0	12
Lady Alice	0.17	0	0.58	0.08	0.08	0.08	0	0	0	0	12
Whatupuke	0.42	0	0.25	0.08	0.17	0.08	0	0	0	0	12
Coppermine	0.17	0	0.25	0	0.17	0	0.25	0.17	0	0	12
Tawhiti Rahi	0	0	1	0	0	0	0	0	0	0	12
Aorangi	0	0	1	0	0	0	0	0	0	0	12
Stack B	0	0	1	0	0	0	0	0	0	0	4
Aorangaia	0	0	1	0	0	0	0	0	0	0	2

Locus 1/C2

Allele	132	144	146	148	150	152	154	156	2n
Brothers	0	1	0	0	0	0	0	0	36
Stephens	0	0.64	0.36	0	0	0	0	0	14
Middle Trio	0	0.92	0.08	0	0	0	0	0	12
North Trio	0	1	0	0	0	0	0	0	6
South Trio	0	1	0	0	0	0	0	0	4
Motunau	0	0.75	0	0.17	0	0.08	0	0	12
Moutoki	0	0	0	0	0.5	0.5	0	0	12
Karewa	0	1	0	0	0	0	0	0	12
Ruamahua-iti	0	0.75	0	0	0.17	0.08	0	0	12
Ruamahua-nui	0	0.17	0.17	0	0	0.5	0.17	0	12
Hongiora	0	0.5	0	0	0.2	0.1	0	0.2	10
Hernia	0	0.33	0.33	0	0	0.17	0.17	0	6
Middle Mercury	0	0.92	0.08	0	0	0	0	0	12
Green Mercury	0	0.83	0	0	0.17	0	0	0	12
Stanley	0	0.25	0.08	0	0.42	0.08	0	0.17	12
Red Mercury	0	0.67	0.33	0	0	0	0	0	6
Cuvier	0	0.63	0	0	0.38	0	0	0	8
Little Barrier	0	0.75	0	0.08	0	0.17	0	0	12
Hen	0.08	0.83	0	0	0	0.08	0	0	12
Lady Alice	0	0.42	0	0	0	0.17	0.42	0	12
Whatupuke	0	0.5	0	0	0	0	0.5	0	12
Coppermine	0	0.75	0	0	0	0.08	0.17	0	12
Tawhiti Rahi	0	0.08	0.92	0	0	0	0	0	12
Aorangi	0	0.17	0.83	0	0	0	0	0	12
Stack B	0	0	1	0	0	0	0	0	4
Aorangaia	0	0	1	0	0	0	0	0	2

27 Table of all samples used in each dataset

Island group	Island	Allozymes	Microsats*	mtDNA*	Source**
Poor Knights	Tawhiti Rahi	FT2105			CHD
		FT2106			CHD
		FT2107			CHD
		FT2108			CHD
		FT2520	TR2520M	TR2520M	CHD
		FT2521	TR2521M	TR2521M	CHD
		FT2522	TR2522M	TR2522M	CHD
		FT2523			CHD
		FT2524	TR2524F		CHD
		FT2525	TR2525F	TR2525F	CHD
		FT2526			CHD
		FT2527			CHD
		FT2528			CHD
		FT2529	TR2529F	TR2529F	CHD
		FT2530			CHD
Poor Knights	Aorangi	FT2030			CHD
		FT2031			CHD
		FT2032			CHD
		FT2043			CHD
		FT2044			CHD
		FT2045			CHD
		FT2046			CHD
		FT2057			CHD
		FT2627			CHD
		FT2629			CHD
		FT2630			CHD
		FT2631			CHD
		FT2632			CHD
		FT2633			CHD
		FT2634			CHD
		FT2635			CHD
		FT2636			CHD
		FT2637			CHD
		FT2638	A2638F	A2638F	CHD
		FT2639	A2639F	A2639F	CHD
			A2640F	A2640F	CHD
			A2643M	A2643M	CHD
			A2645M	A2645M	CHD
			A2647M		CHD
Poor Knights	Aorangaia	FT2619	Aa2619F	Aa2619F	CHD
Poor Knights	Stack B	FT2116			CHD
		FT2535	SB2535F	SB2535F	CHD
		FT2540	SB2540M	SB2540M	CHD
Hen & Chickens	Hen	FT2400			CHD
		FT2405	H2405F	H2405F	CHD
		FT2406	H2406F	H2406F	CHD
		FT2407	H2407F	H2407F	CHD
		FT2409			CHD
		FT2410	H2410M		CHD
		FT2412			CHD
		FT2413	H2413M	H2413M	CHD
		FT2414			CHD
		FT2415			CHD
		FT2417			CHD
		FT2418	H2418M		CHD
		FT2419			CHD
		FT2420			CHD
		FT2421			CHD
Hen & Chickens	Lady Alice	FT2357			CHD

Island group	Island	Allozymes	Microsats*	mtDNA*	Source**
		FT2358			CHD
		FT2359			CHD
		FT2360			CHD
		FT2362			CHD
		FT2363			CHD
		FT2364			CHD
		FT2368			CHD
		FT2441			CHD
		FT2443			CHD
		FT2444			CHD
		FT2445			CHD
		FT2446			CHD
		FT2447			CHD
		FT2448			CHD
			T2M	LAT2M	DML
			T18M	LAT18M	DML
			T20F		DML
			T21F	LAT21F	DML
			T30M		DML
			T34F	LAT34F	DML
Hen & Chickens	Whatupuke	FT2500			CHD
		FT2501			CHD
		FT2502			CHD
		FT2503			CHD
		FT2504			CHD
		FT2505			CHD
		FT2851			CHD
		FT2852			CHD
		FT2853			CHD
		FT2854			CHD
		FT2855			CHD
		FT2856			CHD
		FT2857			CHD
		FT2858			CHD
		FT2859			CHD
			T51M	WT51M	DML
			T54M		DML
			T55		DML
			T56M		DML
			T58M	WT58M	DML
			T101		DML
Hen & Chickens	Coppermine	FT2787	Cp2787M	Cp2787M	CHD
		FT2789			CHD
		FT2790			CHD
		FT2791	Cp2791F	Cp2791F	CHD
		FT2792			CHD
		FT2793			CHD
		FT2794			CHD
		FT2795	Cp2795F	Cp2795F	CHD
		FT2796	Cp2796M		CHD
		FT2797			CHD
		FT2798	Cp2798M	Cp2798M	CHD
		FT2799	Cp2799F		CHD
		FT2800			CHD
		FT2801			CHD
		FT2802			CHD
Little Barrier	Little Barrier	FT2925			CHD
		FT2926			CHD
		FT2927			CHD
		FT2928			CHD

Island group	Island	Allozymes	Microsats*	mtDNA*	Source**
			LB2980M	LB2980M	CHD
			LB2981F	LB2981F	CHD
			LB2982M	LB2982M	CHD
			LB2983F		CHD
			LB3025M	LB3025M	CHD
			LB3027F		CHD
Cuvier	Cuvier	FT2916	C2916M	C2916M	CHD
		FT2917	C2917F	C2917F	CHD
		FT2918	C2918M	C2918M	CHD
		FT2919	C2919M	C2919M	CHD
Mercuries	Red Mercury	FT2653	RM2653M	RM2653M	CHD
		FT2654	RM2654F	RM2654F	CHD
		FT2655	RM2655F		CHD
		FT2960			CHD
		FT2961			CHD
		FT2963			CHD
		FT2964			CHD
		FT2965			CHD
		FT2966			CHD
		FT2968			CHD
		FT2969			CHD
Mercuries	Stanley	FT133			CHD
		FT134			CHD
			Sn2349M	Sn2349M	CHD
			Sn2350M		CHD
		FT2351	Sn2351M	Sn2351M	CHD
		FT2700	Sn2700F		CHD
		FT2701			CHD
		FT2702			CHD
		FT2703	Sn2703F	Sn2703F	CHD
		FT2704	Sn2704F		CHD
		FT2705			CHD
		FT2706			CHD
		FT2707			CHD
		FT2708			CHD
		FT2709			CHD
Mercuries	Middle Mercury	FT102			CHD
		FT103			CHD
		FT104			CHD
		FT106			CHD
		FT107			CHD
		FT108			CHD
		FT2365			CHD
		FT2366			CHD
		FT2367			CHD
		FT2369	MM2369M	MM2369M	CHD
		FT2370			CHD
		FT2371			CHD
		FT2372			CHD
		FT2373	MM2373F	MM2373F	CHD
		FT2374			CHD
		FT2375			CHD
		FT2376			CHD
			MM2720M		CHD
			MM2728F		CHD
			MM2730M		CHD
			MM2742F		CHD
Mercuries	Green Mercury	FT117			CHD
		FT118			CHD
		FT119			CHD

Island group	Island	Allozymes	Microsats*	mtDNA*	Source**
		FT120			CHD
		FT121			CHD
		FT122			CHD
		FT2377	G2377M		CHD
		FT2378	G2378M		CHD
		FT2379	G2379M	G2379M	CHD
		FT2380	G2380F	G2380F	CHD
		FT2381			CHD
		FT2382	G2382F		CHD
		FT2383	G2383F	G2383F	CHD
		FT2384			CHD
		FT2385			CHD
Aldermen	Ruamahua-nui	FT2173	RN2173F	RN2173F	CHD
		FT2174			CHD
		FT2175	RN2175F	RN2175F	CHD
		FT2176	RN2176M	RN2176M	CHD
		FT2177	RN2177F	RN2177F	CHD
		FT2178	RN2178M	RN2178M	CHD
		FT2179	RN2179M	RN2179M	CHD
Aldermen	Ruamahua-iti	FT2149	RI2149U	RI2149	CHD
		FT2150			CHD
		FT2151			CHD
		FT2152			CHD
		FT2154	RI2154M	RI2154M	CHD
		FT2155			CHD
		FT2156			CHD
		FT2157	RI2157F	RI2157F	CHD
		FT2158	RI2158F	RI2158F	CHD
		FT2159	RI2159M	RI2159M	CHD
		FT2160			CHD
		FT2161			CHD
		FT2162	RI2162F	RI2162F	CHD
		FT2163			CHD
		FT2170			CHD
		FT2172			CHD
Aldermen	Hongiora	FT2164	Ho2164M	Ho2164M	CHD
		FT2166	Ho2166F	Ho2166F	CHD
		FT2167	Ho2167M	Ho2167M	CHD
		FT2168	Ho2168M	Ho2168M	CHD
		FT2169	Ho2169M	Ho2169M	CHD
Aldermen	Hernia	FT2180	Hr2180M	Hr2180M	CHD
		FT2182	Hr2182F	Hr2182F	CHD
		FT2185	Hr2185F	Hr2188F	CHD
Karewa	Karewa	FT2069			CHD
		FT2070	K2070F	K2070F	CHD
		FT2071			CHD
		FT2072	K2072F	K2072F	CHD
		FT2073			CHD
		FT2074			CHD
		FT2075	K2075F	K2075F	CHD
		FT2076			CHD
		FT2077			CHD
		FT2078			CHD
		FT2079			CHD
		FT2080			CHD
		FT2081	K2081M	K2081M	CHD
		FT2082			CHD
		FT2083	K2083M	K2083M	CHD
			K2086M	K2086M	CHD

Island group	Island	Allozymes	Microsats*	mtDNA*	Source**
Plate	Motunau	FT2130	Mn2130M	Mn2130M	CHD
		FT2132			CHD
		FT2133			CHD
		FT2134	Mn2134M	Mn2134M	CHD
		FT2135	Mn2135M	Mn2135M	CHD
		FT2136	Mn2136F	Mn2136F	CHD
		FT2137			CHD
			Mn2138F	Mn2138F	CHD
			Mn2139F	Mn2139F	CHD
		FT2141			CHD
		FT2142			CHD
		FT2143			CHD
		FT2144			CHD
		FT2145			CHD
		FT2140			CHD
		FT2146			CHD
		FT2147			CHD
Rurimas	Moutoki	FT2100	Mk2100M	Mk2100M	CHD
		FT2101	Mk2101M	Mk2101M	CHD
		FT2102	Mk2102F	Mk2102F	CHD
			Mk2103M	Mk2103M	CHD
		FT2104			CHD
		FT2105	Mk2105F	Mk2105F	CHD
		FT2106			CHD
		FT2107			CHD
		FT2108			CHD
		FT2109	Mk2109F	Mk2109F	CHD
		FT2110			CHD
		FT2111			CHD
		FT2112			CHD
		FT2113			CHD
		FT2114			CHD
		FT2115			CHD
Stephens	Stephens	CD1343			CHD
		CD1344			CHD
		CD1345			CHD
		CD1346			CHD
		CD1347			CHD
		CD1348			CHD
		CD1349			CHD
		CD1350			CHD
		CD1351			CHD
		CD1352			CHD
		CD1353			CHD
		CD1354			CHD
		CD1355			CHD
		CD1356			CHD
		CD1357			CHD
			4143M	S4143M	DML
			4162M	S4162M	DML
			4172F	S4172F	DML
			4217F	S4212F	DML
				S01F	DML
			05F		DML
				S09M	DML
			10M		DML
			17M	S17M	DML
				S21F	DML

Island group	Island	Allozymes	Microsats*	mtDNA*	Source**
Trios	Middle Trio	FT2000			CHD
		FT2002	MT2002M	MT2002M	CHD
		FT2003	MT2003M	MT2003M	CHD
		FT2004			CHD
		FT2005	MT2005F	MT2005F	CHD
		FT2006			CHD
		FT2007	MT2007F	MT2007F	CHD
		FT2008	MT2008F	MT2008F	CHD
		FT2009	MT2009M		CHD
		FT2010			CHD
		FT2011			CHD
		FT2012			CHD
		FT2013			CHD
		FT2014			CHD
		FT2015			CHD
		FT2017			CHD
		FT2018			CHD
		FT2020			CHD
		FT2022			CHD
		FT2023			CHD
Trios	North Trio	FT2039	NT2039M	NT2039M	CHD
		FT2040	NT2040F	NT2040F	CHD
		FT2041	NT2041M		CHD
Trios	South Trio	FT2049	ST2049M		CHD
		FT2050			CHD
		FT2051	ST2051M	ST2051M	CHD
Brothers	North Brother		B220M	B220M	CHD
			B223F	B223F	CHD
		FT224	B224F	B224F	CHD
		FT225	B225F	B225F	CHD
		FT226			CHD
		FT227			CHD
		FT228	B228M	B228M	CHD
		FT229			CHD
		FT231	B231M	B231M	CHD
		FT232			CHD
		FT233			CHD
		FT234	B234M		CHD
		FT235	B235F	B235F	CHD
		FT236			CHD
		FT237			CHD
			B241M		CHD
			B244M		CHD
			B246F		CHD
			B247M		CHD
			B249F		CHD
			B250M		CHD
			B252M		CHD
			B255F		CHD
			B260F		CHD
			B266F		CHD

*CHD FT and CD labels were renamed with number only, adding prefix to denote island and suffix to denote sex

**CHD=Charles Daugherty; DML=David Lambert

28
29

Maximum likelihood tree of tuatara mtDNA 50 haplotypes. Strict consensus tree of two trees with likelihood score of 1888.8. Tree is rooted between northern and Cook Strait populations.