Genetic affinities of Hochstetter's frog (*Leiopelma hochstetteri*) populations in the Bay of Plenty

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ABSTRACT

Mitochondrial cytochrome b (cyt b) sequence data were used to examine the taxonomic affinities of three populations of Hochstetter's frog (*Leiopelma hochstetteri*) in the Bay of Plenty, New Zealand. Direct sequence comparisons using 600bp of cyt b data show that the Hochstetter's frog populations at Otawa and the Kaimai Range contain four previously undescribed genetic lineages of this endangered frog species. Phylogenetic reconstructions were then used to compare the four unique haplotypes identified in the Bay of Plenty with all 27 haplotypes identified previously during an extensive phylo-geographic analysis of Hochstetter's frog. Our analyses suggest that the Bay of Plenty frogs are genetically distinct from all other known populations of Hochstetter's frog. The Kaimai populations have their closest affinities with frog populations in southern Coromandel, while the frogs in Otawa are most genetically similar to those in the Hunua Ranges and Waikato. However, both Bay of Plenty populations have probably been separated from these other populations of Hochstetter's frog for several thousand years.

Key Words: Hochstetter's frog, *Leiopelma hochstetteri*, genetic affinities, Bay of Plenty.

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1. Introduction

Hochstetter's frog (*Leiopelma hochstetteri*) was the first of the New Zealand native frog species to be formally recognised (Fitzinger 1861) and has been protected since 1922 (Bell 1985, 1994). Although the most abundant of the remaining endemic frogs, *L. hochstetteri* is categorised as 'At Risk' by the Red Data Book categories of the IUCN (International Union for the Conservation of Nature), and has a 'Sparse' rank, under the new Department of Conservation threatened species ranking system (Molloy et al. 2002; Hitchmough 2002).

The species currently lives in fragmented populations across the North Island and on Great Barrier Island (Fig. 1) with the highest population density on the Coromandel Peninsula (Newman 1996). Subfossil remains of *L. hochstetteri* appear throughout the North Island and even on the northern half of the South Island, indicating its range was once much wider than it is now (Worthy 1987).

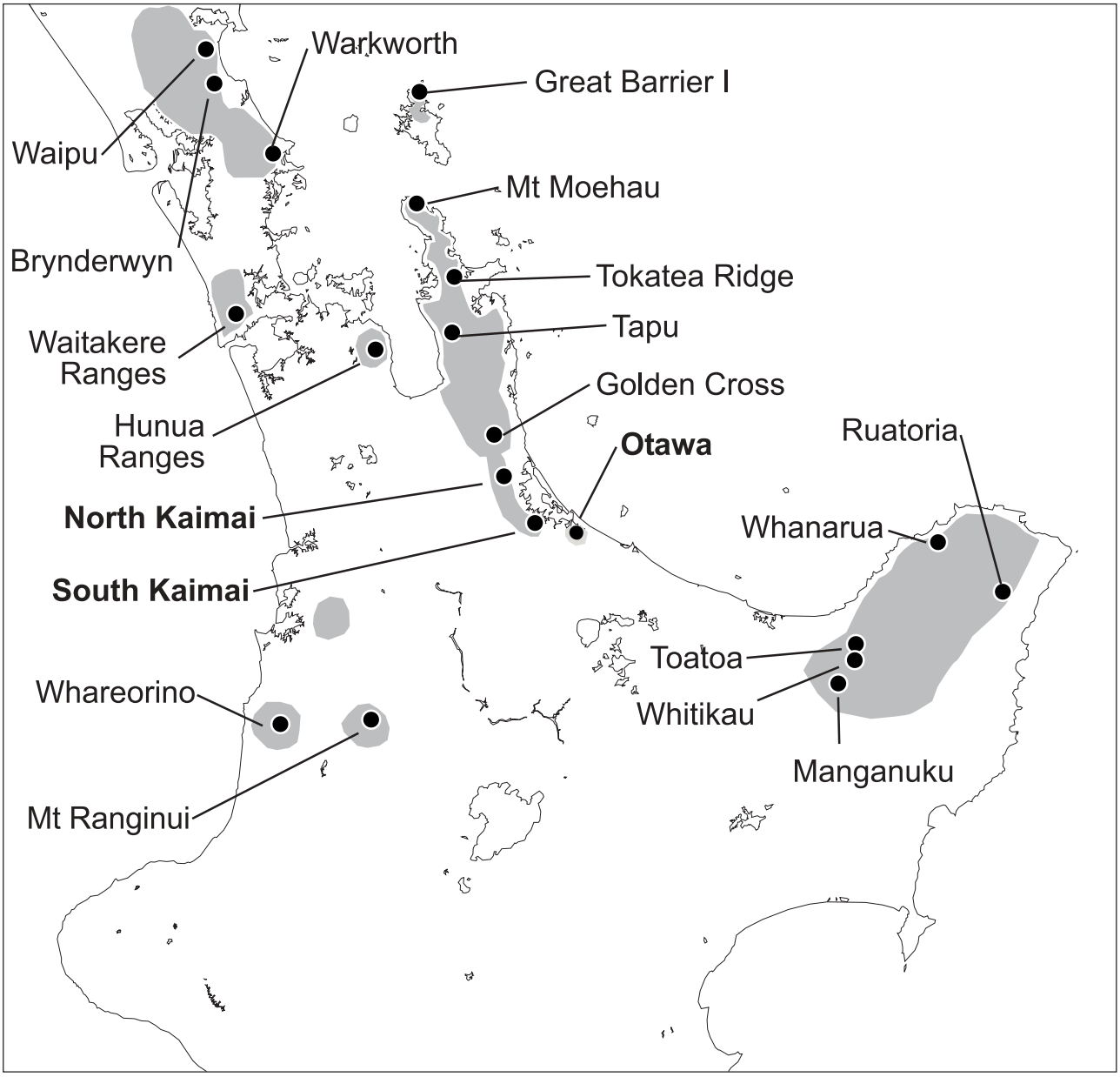

Figure 1. Distribution of *Leiopelma hochstetteri* showing the locality of populations sampled for the current study. The populations sampled from Bay of Plenty Conservancy are in bold. Shaded areas represent the previously known range of these frogs (Newman 1996).

Recently, a new population of *L. hochstetteri* was discovered at Whareorino in the King County region (Thurley & Bell 1994). Our ignorance of an aspect as simple as the distribution of extant populations emphasises the need for more research on this frog.

Cytogenetic work on Hochstetter's frog has previously revealed considerable variation among populations in the average number of supernumerary chromosomes, and in the presence or absence of a unique univalent sex chromosome, which is suggestive of important genetic subdivision and the existence of cryptic species in *L. hochstetteri* (Green 1994). This possibility has been strongly supported by allozyme data (Green 1994) and our recent work using mitochondrial DNA (mtDNA) cytochrome b gene sequence data (Bowsher 2000; N.J. Gemmell et al., pers. comm.). Our studies show up to 4% cyt b sequence difference among populations, with little or no geneflow between geograph-ically close populations, e.g. those in Coromandel.

To date, 67 individuals representing 17 of 19 currently recognised Hochstetter's frog populations have been examined using mtDNA cyt b sequence data. The notable exclusions from this extensive study are populations from the Bay of Plenty region. Within the Bay of Plenty, two distinct Hochstetter's frog populations are known. One is found within the Otawa Scenic Reserve, and the other within the Kaimai Range. All known frog habitat within Otawa Scenic Reserve is currently under threat from quarrying operations.

Our previous work (Bowsher 2000) has shown that *L. hochstetteri* is highly structured at the population level, so it is highly probable that these Bay of Plenty frogs may be evolutionary significant units as yet unidentified for conservation purposes. It was therefore considered imperative that individuals from these populations be included in any comprehensive survey of genetic variability among Hochstetter's frog populations. Consequently, we were asked to extend the molecular genetic work we had undertaken previously (Bowsher 2000) to:

- Determine the genetic uniqueness of the two Hochstetter's frog populations in the Bay of Plenty and
- Determine the degree of relatedness of these populations to all other known populations and comment on the likely origins of these populations.

2. Methods

2.1 SAMPLES

Total genomic DNA was obtained for each of the samples listed in Table 1 using a modification of the Chelex protocol (Walsh et al. 1991). Approximately 2 mm^2 of tissue was suspended in 300 ml of digestion buffer containing 5% Chelex. Proteinase K and RNase were added to final concentrations of 100 mg/ml and the samples were incubated overnight at 37°C. The samples were centrifuged at 13,000 rpm to precipitate debris. The supernatant was transferred to a fresh tube and an equal volume of 5% Chelex in TE added. The sample was centrifuged once more at 13,000 rpm, and the supernatant removed and stored at –20°C.

2.2 PCR AMPLIFICATION AND SEQUENCING

Amplification of mitochondrial cyt b gene partial sequences was achieved by Polymerase Chain Reaction (PCR) using primers designed using comparative sequence alignments (Bowsher 2000) and modified to include T7 and T3 tails to enable direct fluorescent sequencing: cyt b, JB1F+T7 5'-GTAATACGACTCACTA TAGGGCATGAAACTTCGGCTCTCTTMRGG-3', JB36R+T35'-AATTAACCCTCAC TAAAGGGTCTTCTACTGGTTGACCTCCAATTCA-3'. PCRs were carried out in 50 µl reaction mixtures containing 50 ng of template DNA, 10 pmol of each primer, 5 nmol of each dNTP, 5 µl of 10× reaction buffer (500 mM KCl, 100 mM Tris-HCl, pH 9.0), 1.5 mM $MgCl_2$ and 1 unit of *Taq* polymerase (Roche). All reactions were denatured for 2 min at 95°C prior to initiation of the PCR. For all cyt b reactions, the cycling parameters were 35 cycles of 95°C/30 sec, 60°C/ 30 sec, and 72°C/45 sec, followed by a final extension step of 72°C/4 min.

Following amplification, the integrity and size of PCR products were examined using agarose gel electrophoresis and the products were purified by precipitation with isopropanol to remove residual primers and dNTPs. PCR products were sequenced using infrared labelled Licor IRD 800 T7 or T3 promoter primers with a Thermosequenase cycle sequencing kit (Amersham

TABLE1. SAMPLES USED IN THIS STUDY.

SPECIES	REGION	LOCATION	SAMPLE SIZE (n)
Leiopelma archeyi	Coromandel	Tapu[1]	1
	Waikato	Whareorino[2]	1
Leiopelma hochstetteri	Northland	Brynderwyn	3
		Waipu	3
		Warkworth	2
	Auckland	Hunua Ranges	5
		Waitakere Ranges	6
	Coromandel	Golden Cross	5
		Great Barrier Island	6
		Mt Moehau	5
		Tapu	3
		Tokatea Ridge	5
	Bay of Plenty	Kaimai, North[4]	3
		Kaimai, South[4]	3
		Otawa[4]	4
	East Cape	Manganuku[3]	2
		Ruatoria	4
		Toatoa	2
		Whanarua	1
		Whanarua[3]	4
		Whitikau	2
	Waikato	Mt Ranginui	4
		Whareorino	2
		Whareorino[2]	2

[1] Samples collected by B. Waldman

[2] Samples collected by K. Eggers

[3] Samples collected by N.J. Gemmell and J.H. Bowsher

[4] Samples collected by J. Heaphy (DOC Tauranga)

All other samples collected by D.M. Green

Pharmacia Biotech). The reaction conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C/30 sec, 55°C/30 sec, 70°C/1 min, and 10 cycles of 95°C/30 sec, 72°C/1 min. For each individual, several PCR products were sequenced in both directions to ensure sequence fidelity. All sequencing reactions were run on a Licor automated sequencer and analysed using the Base ImagIR software (Licor corporation).

2.3 PHYLOGENETIC ANALYSIS

Individual sequences were aligned using Clustal W and the default gap penalties (Thompson et al. 1994). Identical sequences identified after alignment were then filtered and collapsed in MacClade v3.06 (Maddison & Maddison 1996). Cytochrome b sequences from two Archey's frogs (*Leiopelma archeyi*) were obtained from a previous study (Holyoake et al. 2001) for use as outgroups in our analyses. Maximum likelihood (ML) phylogenetic analyses with and without outgroups were performed on this data set using the PAUP* package (Swofford 2000). ML models and parameters were determined using Modeltest 3.06, which suggested that the Tamura-Nei model with invariant sites (TrN+I) was the optimal model for our rooted trees while a Transition Model including invariant sites (TIM+I) was optimal for our unrooted analyses (Posada & Crandall 1998). Taxa were added randomly for both ML and bootstrap analyses (Felsenstein 1985). For bootstrap analyses, 100 replicates were performed within PAUP to provide an estimate of the statistical significance of the tree topologies generated.

3. Results

Sequence data were obtained from 75 Hochstetter's frogs representing all 19 known populations. When aligned and double-checked for accuracy, at least 600 bps were obtained from every individual and the level of sequence difference among populations ranged from 0 to 3%. None of the sequences obtained appear to be pseudogene-derived because all inter-sequence comparisons show transition/transversion ratios consistent with those previously reported for mtDNA (Lopez et al. 1997). The aligned sequence data obtained for the 10 frogs sampled from the three Bay of Plenty sites, along with representative sequences from other sites, are shown in Appendix 1.

Twenty-eight distinct haplotypes were identified, four of which were found in the three Bay of Plenty populations (Appendix 1, Figs 2, 3). With the exception of two haplotypes, the Northland haplotype common to the Brynderwyn, Waipu and Warkworth populations and the Whanarua haplotype that was also found in one frog from Ruatoria State Forest, no sharing of haplotypes was observed between populations. This strong haplotypic differentiation between sites strongly suggests a lack of contemporary interconnectiveness among populations of Hochstetter's frogs.

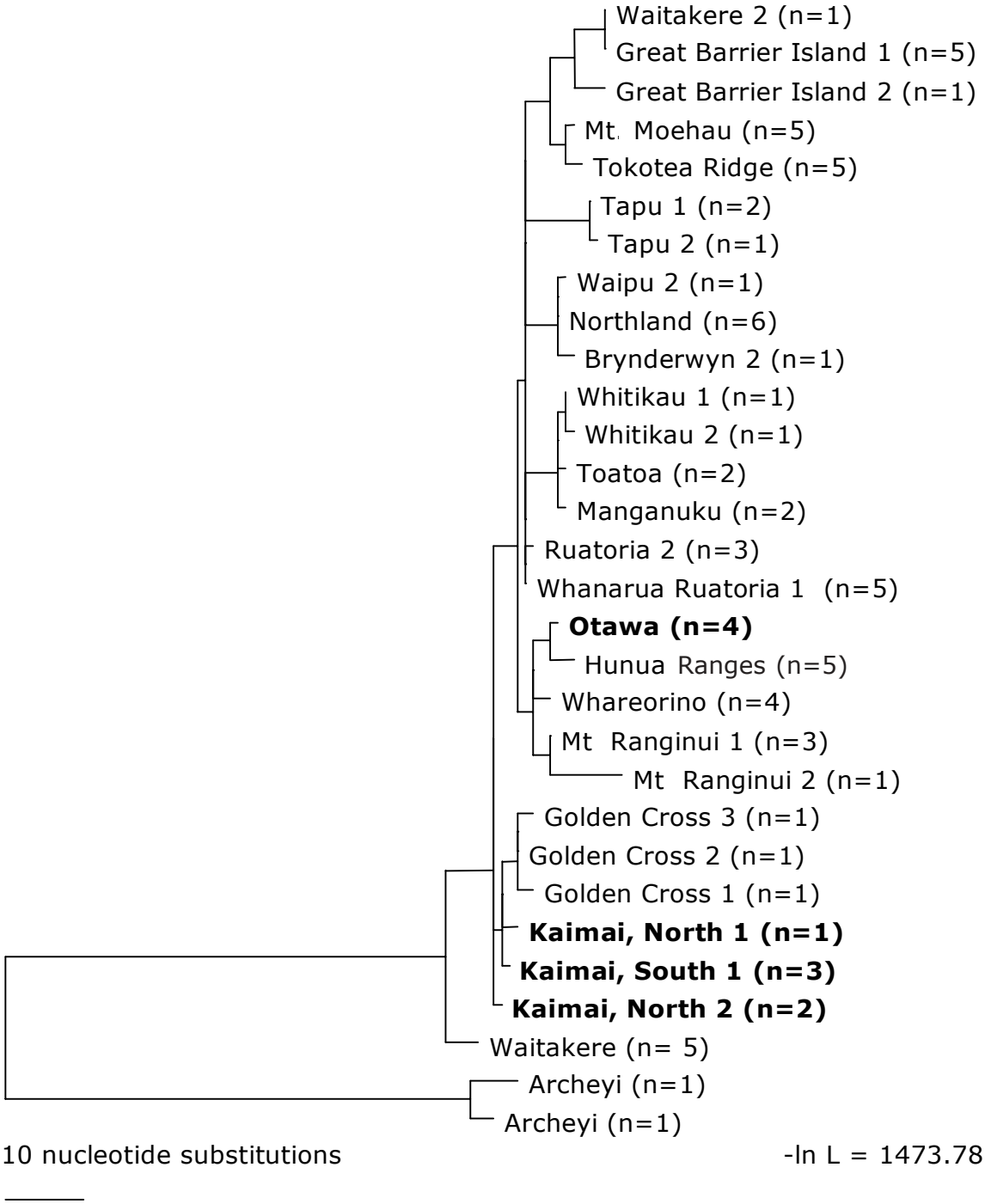

Figure 2. Phenogram rooted by outgroup illustrating the relationships within *L. hochstetteri* based on the 600bp of cyt b gene sequence. The tree was constructed in PAUP* (Swofford 2000) using maximum likelihood analysis. The numbers of individuals (n) represented by each mtDNA haplotype are shown to the right of the haplotypic description. Taxa in bold are populations in the Bay of Plenty Conservancy.

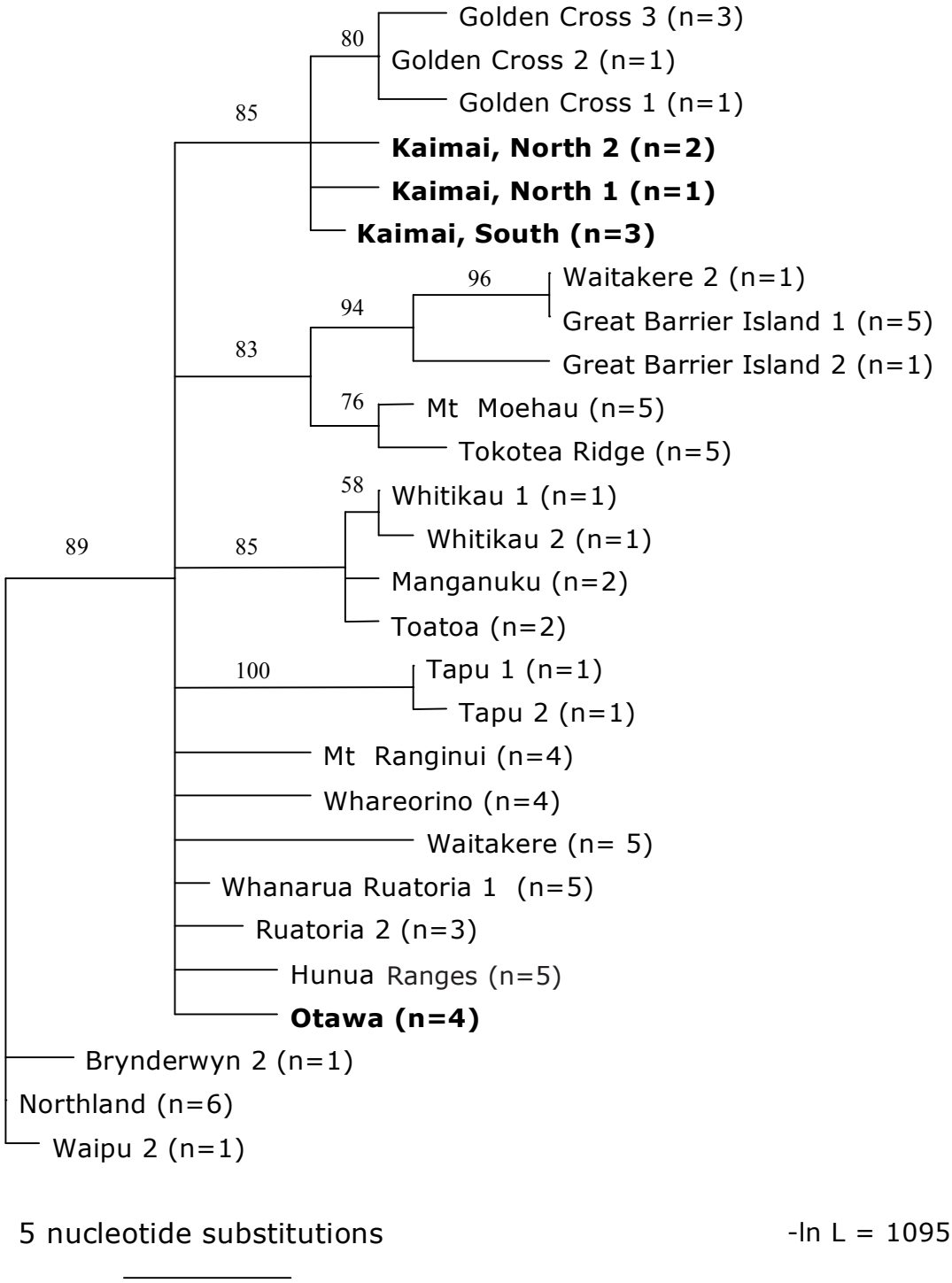

Figure 3. Unrooted phenogram illustrating the relationships within *L. hochstetteri* based on the 600bp of cyt b gene sequence. The tree was constructed in PAUP* (Swofford 2000) using maximum likelihood analysis. Numbers above branches are the proportion of bootstrap replicates where the same branching order was recovered. Branches with bootstrap values of less than 50% were collapsed. The numbers of individuals (n) represented by each haplotype are shown to the right of the haplotypic description. Taxa in bold are populations in the Bay of Plenty Conservancy.

The phylogenetic relationships among populations are incompletely resolved based upon bootstrap confidence values (Fig. 3); nevertheless, a number of strong trends emerge from the data. First, monophyly of Hochstetter's frog was strongly supported in all the phylogenetic analyses performed (Fig. 2). Second, within Hochstetter's frog four well-resolved monophyletic groups (clades) were identified with bootstrap confidence > 80% that correspond well to geographic locality. These clades consisted of populations in South Coromandel / Bay of Plenty, North Coromandel / Great Barrier Island, East Cape, and Northland (Table 1, Fig. 3).

The phylogeographic relationships of the remaining 8 populations (Hunua Ranges, Mt Ranginui, Otawa, Ruatoria, Tapu, Waitakere Ranges, Whareorino, and Whanarua) were not well resolved (bootstrap < 50%), most likely as a consequence of an insufficient number of informative sites in our sequence data. However, with the exception of a shared haplotype among the Whanarua and Ruatoria populations, each of these unresolved populations possessed a unique haplotype that was fixed for that population. It is likely, therefore, that additional sequence data would strengthen the resolution of relationships observed in Fig. 2, in which 5 of the 8 unassigned populations form a weakly supported clade (bootstrap values not shown). This incompletely resolved clade consists of frog populations from the Hunua Ranges, Otawa, Mt Ranganui, and Whareorino. The bootstrap support for this clade is weak (28%), but within the clade the relationships between the populations are stronger. The Hunua Ranges and Otawa haplotypes group together with 48% bootstrap support and Mt Ranganui and Wharerino haplotypes group together with 40% bootstrap support. This relationship might strengthen if more sequence data were available.

4. Discussion

Hochstetter's frog is New Zealand's most widely distributed endemic frog species, being found throughout the North of the North Island. We have analysed 600bp of partial cytochrome b gene sequence from 75 frogs representing all 19 known populations of this species (Fig. 1, Table 1). We have found a very high degree of haplotypic differentiation between populations, with almost every population examined possessing a distinct haplotype. Pairwise nucleotide differences between haplotypes ranged from 0 to 3%. In contrast to this striking variability among populations, the haplotypes identified within a population were highly uniform, with most populations being fixed for one haplotype. Some phylogeographic structure was detected with four well-supported clades identified (South Coromandel / Bay of Plenty, North Coromandel / Great Barrier Island, East Cape, and Northland) that correlated strongly with geography (Figs 2, 3). The exact relationships among all populations could not be resolved with the available data, but it is likely that with more data the ambiguous relationships remaining among some populations could be easily resolved.

The phylogenetic trees obtained from our mitochondrial sequence data suggest that there has been some level of historic interconnectiveness between populations in geographic proximity, but that most of these populations are now isolated from each other and evolving independently. The absence of shared haplotypes between populations supports the view that there is no, or at the most, only very low levels of contemporary interconnectiveness between the remaining populations of Hochstetter's frog, and this view has also been strongly supported by earlier studies using allozyme and karyotypic data (Green 1994).

Our findings have important implications for the development of management plans to ensure the conservation of these frogs. In recent years the importance of maintaining the evolutionary potential of species has been formally recognised with the adoption of 'evolutionarily significant units' (ESUs) to protect historically isolated, genetically distinct, assemblages of a biological species (Ryder 1986; Waples 1991; Moritz 1994). An ESU is a population that is isolated from other conspecific population units, and it embodies an important component of the evolutionary legacy of the species. Moritz (1994) suggests that 'ESUs should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci'. While the genetic criteria for recognising ESUs may be overly restrictive, they can be applied with consistency and offer the advantage of being theoretically sound (Moritz 1994). Most importantly, ESUs avoid the issue of 'how much divergence is enough?' by considering the pattern rather than the amount of genetic divergence.

For the purposes of modern conservation management, all of the 19 known populations of *L. hochstetteri* examined, with the exception of the Northland populations, warrant conservation as independent units for management purposes (Figs 2, 3). The average level of mtDNA divergence between these populations is 1.9% (range 0.17% to 3.99%, S.D 0.7%) at cyt b, which is considered high within a species (Avise 1997), and there is reciprocal monophyly between these populations at mtDNA loci (Figs 2, 3). Further, there are fixed and allele frequency differences at nuclear allozyme loci as well as karyotypic variability known for many of these populations (Green 1994). This striking pattern of genetic differentiation, together with their allopatry, suggests that for conservation management purposes, *L. hochstetteri* populations should be considered as separate ESUs.

Of course, conservation management decisions need to be based on broader considerations, and a major dilemma facing conservation biologists is the question of whether we should conserve more populations because they are genetically distinct, or promote the maintenance of genetically more diverse species. Augmenting this dilemma are the problems of demography and it is clear that preserving genetically distinctive species may prove of little value if the population size has dropped below a critical level (Lande 1993; Lynch et al. 1995). In resolving the dilemma, we cannot afford to lose sight of the reality that resources available to managers are limited. Ensuring the viability of ESUs, even if possible, might come at the cost of sacrificing other potentially more important projects such as those designed to detect and conserve cryptic but genetically distinct species.

Joint management to preserve the species may best serve the maintenance of diversity and continued population viability. The alternative strategy—separate management—places what remaining diversity exists in significant jeopardy because for many of these frog populations the demographic concerns may considerably outweigh those of genetics. However, in the absence of accurate census data we are not yet in a position to determine the best course of action for the future management of *L. hochstetteri*.

5. Conclusion

The management questions that were originally posed can be readily addressed with the available data. The Kaimai and Otawa populations of frogs found in the Bay of Plenty are genetically unique, with four previously undescribed haplotypes identified in these populations. Three haplotypes were found in the 6 frogs sampled from the Kaimai Range, while one haplotype was identified in the four frogs sourced from Otawa. While each of these four haploypes was unique, the Kaimai frogs showed their closest genetic affinities to haplotypic lineages present in Golden Cross, south Coromandel. This relationship was strongly supported, being observed in 85% of our bootstrap replicates. The relationship of the Otawa frogs was more ambiguous. The haplotype identified to the four frogs sampled from this locality is unique among all the populations examined, but its closest affinities were to Hunua, Kaimai or Waikato frogs. Additional sequence data would be needed to resolve these relationships further; however, it is extremely unlikely that the Otawa frogs have strong affinities with populations outside of the Hunua Ranges or the Waikato. However, despite their ambiguous affinities, the Otawa frogs are unique and, at present, should be considered important evolutionary lineages that need to be protected for the conservation of this species.

Further research using nuclear genetic markers is warranted if we wish to document more fully the relationships among the remaining populations of *L. hochstetteri,* particularly those of the Otawa frogs. Such data would provide additional resolution of the phylogenetic relationships among these populations. Furthermore, because nuclear DNA markers are biparentally inherited (unlike mtDNA which is maternally inherited), they would also provide useful tools for examining population variability (He), effective population size (Ne), male and female gene flow and, potentially, individual reproductive success data that will be increasingly important if we are to not only set priorities and goals for native frog conservation but also monitor outcomes.

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Appendix 1

ALIGNED PARTIAL cyt b GENE SEQUENCES

The aligned sequences presented are those obtained from frogs from the Bay of Plenty populations as well as representative sequences obtained from frog populations in Coromandel, East Cape, Northland and the Hunua Ranges. The Asterisk (*) below the alignment shows conserved base positions.

```
                    10        20        30        40        50        60
                    ----|----|----|----|----|----|----|----|----|----|----|--
NorthKaimai1        CACACATATTTGCCGGGACGTTAACTACGGGTGATTAATCCGAAATATCCATGCCAACGGAG
NorthKaimai2        CACACATATTTGCCGGGACGTTAACTACGGGTGATTAATCCGAAATATCCATGCCAACGGAG
SouthKaimai         CACACATATTTGCCGGGACGTTAACTACGGGTGATTAATCCGAAATATCCATGCCAACGGAG
Otawa               CGCACATATTTGCCGGGACGTTAACTACGGGTGATTAATCCGAAATATCCATGCCAACGGAG
GoldenCross1        CGCACATATTTGCCGGGACGTTAACTACGGGTGATTAATCCGAAATATCCATGCCAACGGAG
GoldenCross2        CGCAC-TATTTGCCGGGACGTTAACTACGGGTGATTAATCCGAAATATCCATGCCAACGGAG
GoldenCross3        CGCACATATTTGCCGGGACGTTAACTACGGGTGATTAATCCGAAATATCCATGCCAACGGAG
Northland           CACAC-TATTTGCCGG-AC---AACTACGGGTGATTAATCCGAAATATCCATGCCAACGGAG
Manganuku           CACACATATTTGCCGGGACGTTAACTACGGGTGGTTAATCCGAAATATCCATGCCAACGGAG
Hunua               CGCACATATTTGCCGGGACGTTAACTACGGGTGATTAATCCGAAATATCCATGCCAACGGAG
Archeyi             CACACACATCTGCCGAGATGTCAACTGCGGATGACTAATCCGAAATATGCATGCCAACGGGG
                    * ***  ** *****  *    **** *** **  ************* *********** *

                          70        80        90       100       110       120
                    --|----|----|----|----|----|----|----|----|----|----|----|----
NorthKaimai1        CTTCTTTATTTTTCATTTGTATTTATTTACATATCGGACGAGGCATATACTACGGATCCTAC
NorthKaimai2        CTTCTTTATTTTTCATTTGKATTTATTTACATATCGGACGAGGCATATACTACGGATCCTAC
SouthKaimai         CTTCTTTATTTTTCATTTGKATTTATTTACATATCGGACGAGGCATATACTACGGATCCTAC
Otawa               CTTCTTTATTTTTCATTTGKATTTATTTACATATCGGACGAGGCATATACTACGGATCCTAC
GoldenCross1        CTTCTTTATTTTTCATTTGTATTTATTTACATATCGGACGAGGCATATACTACGGATCCTAC
GoldenCross2        CTTCTTTATTTTTCATTTGTATTTATTTACATATCGGACGAGGCATATACTACGGATCCTAC
GoldenCross3        CTTCTTTATTTTTCATTTGTATTTATTTACATATCGGACGAGGCATATACTACGGATCCTAC
Northland           CTTCTTTATTTTTCATTTGTATTTATTTACATATCGGACGAGGCATATACTACGGATCCTAC
Manganuku           CTTCTTTATTTTTCATTTGTATTTATTTACATATCGGACGAGGCATATACTACGGATCCTAC
Hunua               CTTCTTTATTTTTCATTTGTATTTATCTACATATCGGACGAGGCATATACTACGGATCCTAC
Archeyi             CCTCACTTTTCTTCATTTGCATTTACCTGCACATCGGACGCGGCATGTACTACGGATCTTAC
                    * **  * ** ******** *****  * ** ******** ***** *********** ***

                       130       140       150       160       170       180
                    |----|----|----|----|----|----|----|----|----|----|----|----|-
NorthKaimai1        CTATTCAAAGAAACATGAAATATTGGCGTAGCCCTTTTATTTTTAGTTATAGCAACAGCCTT
NorthKaimai2        CTATTCAAAGAAACATGAAATATTGGCGTAGCCCTTTTATTTTTAGTTATAGCAACAGCCTT
SouthKaimai         CTATTCAAAGAAACATGAAATATTGGCGTAGCCCTTTTATTTTTAGTTATAGCAACAGCCTT
Otawa               CTATTCAAAGAAACATGAAATATTGGCGTAGCCCTTTTATTTTTAGTTATAGCAACAGCCTT
GoldenCross1        CTATTCAAAGAAACATGAAATATTGGCGTA--CCTTTTATTTTTAGTTATAGCAACAGCCTT
GoldenCross2        CTATTCAAAGAAACATGAAATATTGGCGTA--SCTTTTATTTTTAGTTATAGCAACAGCCTT
GoldenCross3        CTATTCAAAGAAACATGAAATATTGGCGTA--CCTTTTATTTTTAGTTATAGCAACAGCCTT
Northland           CTATTCAAAGAAACATGAAATATTGGCGTA--CCTTTTATTTTTAGTTATAGCAACAGCCTT
Manganuku           CTATTCAAAGAAACATGAAATATTGGCGTA--CCTTTTATTTTTAGTTATAGCAACAGCCTT
Hunua               CTATTCAAAGAAACATGAAGTATTGGCGTA--CCTTTTATTTTTAGTTATAGCAACAGCCTT
Archeyi             CTGTTCAAAGAAACATGAAATATCGGCGTCGTCCTATTATTTCTAGTTATAGCAACAGCCTT
                    ** ***************** *** *****    ** ****** ********************
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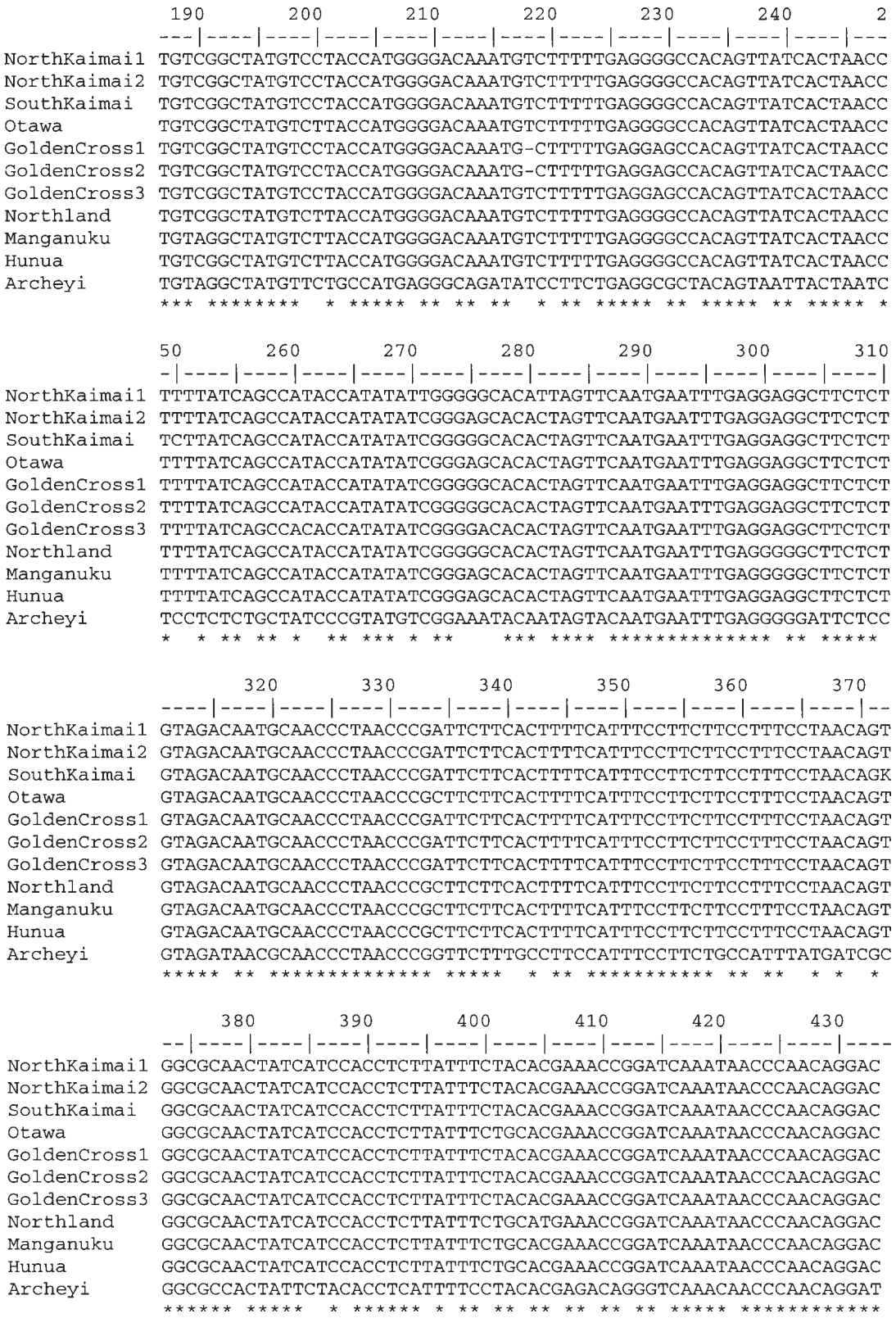
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SouthKaimai TGTCGGCTATGTCCTACCATGGGGACAAATGTCTTTTTGAGGGGCCACAGTTATCACTAACC
Otawa TGTCGGCTATGTCTTACCATGGGGACAAATGTCTTTTTGAGGGGCCACAGTTATCACTAACC
GoldenCross1 TGTCGGCTATGTCCTACCATGGGGACAAATG-CTTTTTGAGGAGCCACAGTTATCACTAACC
GoldenCross2 TGTCGGCTATGTCCTACCATGGGGACAAATG-CTTTTTGAGGAGCCACAGTTATCACTAACC
GoldenCross3 TGTCGGCTATGTCCTACCATGGGGACAAATGTCTTTTTGAGGAGCCACAGTTATCACTAACC
Northland TGTCGGCTATGTCTTACCATGGGGACAAATGTCTTTTTGAGGGGCCACAGTTATCACTAACC
Manganuku TGTAGGCTATGTCTTACCATGGGGACAAATGTCTTTTTGAGGGGCCACAGTTATCACTAACC
Hunua TGTCGGCTATGTCTTACCATGGGGACAAATGTCTTTTTGAGGGGCCACAGTTATCACTAACC
Archeyi TGTAGGCTATGTTCTGCCATGAGGGCAGATATCCTTCTGAGGCGCTACAGTAATTACTAATC
50 260 270 280 290 300 310
NorthKaimai1 TTTTATCAGCCATACCATATATTGGGGGCACATTAGTTCAATGAATTTGAGGAGGCTTCTCT
NorthKaimai2 TTTTATCAGCCATACCATATATCGGGAGCACACTAGTTCAATGAATTTGAGGAGGCTTCTCT
SouthKaimai TCTTATCAGCCATACCATATATCGGGGGCACACTAGTTCAATGAATTTGAGGAGGCTTCTCT
Otawa TTTTATCAGCCATACCATATATCGGGAGCACACTAGTTCAATGAATTTGAGGAGGCTTCTCT
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Manganuku TTTTATCAGCCATACCATATATCGGGAGCACACTAGTTCAATGAATTTGAGGGGGCTTCTCT
Hunua TTTTATCAGCCATACCATATATCGGGAGCACACTAGTTCAATGAATTTGAGGAGGCTTCTCT
Archeyi TCCTCTCTGCTATCCCGTATGTCGGAAATACAATAGTACAATGAATTTGAGGGGGATTCTCC
320 330 340 350 360 370
NorthKaimai1 GTAGACAATGCAACCCTAACCCGATTCTTCACTTTTCATTTCCTTCTTCCTTTCCTAACAGT
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Hunua GTAGACAATGCAACCCTAACCCGCTTCTTCACTTTTCATTTCCTTCTTCCTTTCCTAACAGT
Archeyi GTAGATAACGCAACCCTAACCCGGTTCTTTGCCTTCCATTTCCTTCTGCCATTTATGATCGC
380 390 400 410 420 430
NorthKaimai1 GGCGCAACTATCATCCACCTCTTATTTCTACACGAAACCGGATCAAATAACCCAACAGGAC
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Hunua GGCGCAACTATCATCCACCTCTTATTTCTGCACGAAACCGGATCAAATAACCCAACAGGAC
Archeyi GGCGCCACTATTCTACACCTCATTTTCCTACACGAGACAGGGTCAAACAACCCAACAGGAT

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                   440        450        460        470        480        490
                   |----|----|----|----|----|----|----|----|----|----|----|----|-
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NorthKaimai2       TAAACTCAAACCCTGATAAAGTCCCCTTCCACCCATATTTCTCTTACAAAGACTTACTAGGC
SouthKaimai        TAAACTCAAACCCTGATAAAGTCCCCTTCCACCCATATTTCTCTTACAAAGACTTACTAGGC
Otawa              TAAACTCAAACCCTGATAAAGTCCCCTTCCACCCATATTTCTCTTACAAAGACTTACTAGGC
GoldenCross1       TAAACTCAAACCCTGATAAAGTCCCCTTCCACCCAAATTTCTCTTACAAAGACTTACTAGGC
GoldenCross2       TAAACTCAAACCCTGATAAAGTCCCCTTCCACCCATATTTCTCTTACAAAGACTTACTAGGC
GoldenCross3       TAAACTCAAACCCTGATAAAGTCCCCTTCCACCCATATTTCTCTTACAAAGACTTACTAGGC
Northland          TAAACTCAAACCCTGATAAAGTCCCCTTCCACCCATATTTCTCTTACAAAGACTTACTAGGC
Manganuku          TAAACTCAAACCCTGATAAAGTCCCCTTCCACCCATATTTCTCTTACAAAGACTTACTAGGC
Hunua              TAAACTCAAACCCTGATAAAGTCCCCTTCCACCCATATTTCTCTTACAAAGACTTACTAGGC
Archeyi            TAAACTCAAATCCTGACAAAGTAACTTTCCACCCCTATTTTTCCTATAAAGACCTCCTAGGC
                   ********** ***** *****  * ********  **** ** ** ****** * ******

                    500        510        520        530        540        550        5
                   ---|----|----|----|----|----|----|----|----|----|----|----|---
NorthKaimai1       TTCTACATTATAATCACCTCCATATGCTTACTAGCCCTATTTGCCCCAAATCTTCTAGGAGA
NorthKaimai2       TTCTACATCATAATCACCTCCATATGCTTACTAGCCCTATTTGCCCCAAATCTTCTAGGAGA
SouthKaimai        TTCTACATTATAATCACCTCCATATGCTTACTAGCCCTATTTGCCCCAAATCTTCTAGGAGA
Otawa              TTCTACATTATAATCACCTCCATATGCTTACTAGCCCTATTTACCCCAAATCTTCTAGGAGA
GoldenCross1       TTCTACATTATAATCACCTCCATATGCTTACTAGCCC-ATTTGCCCCAAATCTTCTAGGAGA
GoldenCross2       TTCTACATTATAATCACCTCCATATGCTTACTAGCCCTATTTGCCCCAAATCTTCTAGGAGA
GoldenCross3       TTCTACATTATAATCACCTCCATATGCTTACTAGCCCTATTTGCCCCAAATCTTCTAGGAGA
Northland          TTCTACATTATAATCACCTCCATATGCCTACTAGCCCTATTTGCCCCAAATCTTCTAGGAGA
Manganuku          TTCTACATTATAATCACCTCCGTATGCTTACTAGCCCTATTTGCCCCAAATCTTCTAGGAGA
Hunua              TTCTACATTATAATCACCTCCATATGCTTACTAGCCCTATTTGCCCCAAATCTTCTAGGAGA
Archeyi            TTCTACATAATAATTGTTACCCTGGGCCTTCTAGCTTTATTTTCCCCAAACCTCTTAGGAGA
                   ******** *****     ** *  ** * *****   **** ******* **  *******

                   60         570        580        590
                   -|----|----|----|----|----|----|----|----
NorthKaimai1       CCCAGAAAATTTTACCCCTGCCGATCCACTAGTTACTCCAC
NorthKaimai2       CCCAGAAAATTTTACCCCTGCCGATCCACTAGTTACTCCAC
SouthKaimai        CCCAGAAAATTTTACCCCTGCCGATCCACTAGTTACTCCAC
Otawa              CCCAGAAAATTTTACCCCTGCCGATCCACTAGTTACCCCAC
GoldenCross1       CCCAGAAAATTTTACCCCTGCCGATCCACTAGTAACTCCAC
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Hunua              CCCAGAAAATTTTACCCCTGCCGATCCACTAGTTACTCCAC
Archeyi            CCCAGAAAATTTCACCCCTGCAAACCCATTAATTACCCCAC
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