Molecular systematics and conservation of kakariki (*Cyanoramphus* spp.)

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CONTENTS

Molecular systematics and conservation of kakariki (*Cyanoramphus* spp.)

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ABSTRACT

The systematics of the New Zealand endemic parakeet genus *Cyanoramphus* (Maori name: kakariki) has been revised several times over the past 150 years without much success. We use modern molecular techniques (DNA sequence of mitochondrial cytochrome *b* and control region) to address the taxonomic, evolutionary and conservation issues of this genus in detail. Evolutionary trees based on DNA sequences for up to 73 individuals, representing ten taxa, are used in conjunction with the Phylogenetic Species Concept to produce a new classification scheme which recognises ten species (*C. zelandicus, C. ulietanus, C. saisetti, C. cooki, C. malherbi, C. novaezelandiae, C. forbesi, C. unicolor, C. erythrotis, C. auriceps*) and four subspecies (*C. n. chathamensis, C. n. cyanurus, C. n. subflavescens, C. e. hochstetteri*). Based on molecular and geological evidence, we propose that the ancestor of *Cyanoramphus* dispersed from New Caledonia to New Zealand via Norfolk Island in the last 500 000 years prior to colonisation of the sub-antarctic and offshore islands of New Zealand. A detailed colonisation pathway is presented along with taxonomic discussions for each of the species concerned. We also establish *Cyanoramphus* as one of the many ancient links between the biota of New Zealand and New Caledonia. The conservation implications of this study are wide-ranging and will affect the way some of these birds are managed based on their species status and origins.

Keywords: *Cyanoramphus*, kakariki, parakeets, systematics, conservation, molecular evolution, speciation, phylogenetic analysis, molecular clock.

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1. Introduction

Recent ecological, morphological and taxonomic studies (Taylor 1975; Fleming 1979; Nixon 1982; Taylor 1985; Taylor et al. 1986; Greene 1988; Pickard 1990; Elliott et al. 1996; Taylor 1998; Greene 1999) have provided much insight into the biology of *Cyanoramphus,* but comprehensive taxonomic studies of this genus have been few. Triggs & Daugherty (1996) used allozyme electrophoresis data to analyse the relationship of some parakeet species but comparisons with most of the taxa in this genus have not been previously available. Virtually all *Cyanoramphus* taxa are greatly reduced in their distribution, vulnerable, on the verge of extinction or extinct. To enable the most effective conservation actions to be taken, the systematics and taxonomy of these birds need to be updated from their pre-1900 origins in order to provide conservation managers with an accurate view of the fundamental 'management units' (Moritz 1994) of this genus.

Using molecular genetic techniques we infer evolutionary histories (phylogenies) of these birds, supply a revised taxonomic list of the taxa based on molecular phylogenetics and suggest possible future research requirements (see also Boon et al. 2000a & b, and in press). We also provide a quantitative measure of the genetic relationships of the *Cyanoramphus* taxa and use the results of our analyses to review the management priorities for this genus.

2. Background

2.1 THE *Cyanoramphus* GENUS

With an apparent centre of origin in New Zealand, *Cyanoramphus* are believed to have dispersed across ocean barriers to many islands in the South Pacific (Fleming 1976). The distribution of this group of parakeets is from the Society Islands (Raiatea, 16°50'S, 151°30'E; Tahiti, 17°40S, 149°27'W) to the sub-antarctic Macquarie Island (54°30'S, 158°57'E) and from Lord Howe Island (31°28'S, 159°03'E) to the Chatham Islands (43°53'S, 176°33'W). Their range has since contracted due to extinction of four insular endemic taxa (Table 1 & Fig. 1). There were five recognised species (Forshaw & Cooper 1989; Higgins 1999) and numerous subspecies in this genus.

Due to the high degree of differentiation that has occurred on isolated islands, some forms of *Cyanoramphus* have very limited distributions and are thus vulnerable to extinction (Taylor 1985). Although the New Zealand red-crowned parakeet (*C. novaezelandiae*) is rare on the North and South Islands of New Zealand, it is still common on Stewart Island and many offshore (Elliott et al. 1996). Further species and subspecies of red-crowned parakeet occur in New Caledonia, Kermadec, Norfolk, Macquarie, Antipodes and Chatham Islands. The Antipodes Island (green) parakeet (*C. unicolor*) is endemic to the sub-antarctic Antipodes Island, while the only known populations of orange-fronted parakeet

TABLE 1. SPECIES AND SUBSPECIES OF *Cyanoramphus* PARAKEETS AND THEIR DISTRIBUTIONS (TAYLOR 1975; FORSHAW & COOPER 1989; KEARVELL 1998; HIGGINS 1999).

COMMON NAME	SCIENTIFIC NAME*	DISTRIBUTION/RANGE
Chatham Island red-crowned parakeet (CtRCP)	*C. n. chathamensis*	Chatham Islands, New Zealand
New Zealand Red-crowned parakeet (RCP)	*C. novaezelandiae novaezelandiae*	North and South Islands of New Zealand, Stewart and Auckland Islands, many other offshore Islands of New Zealand
Orange-fronted parakeet (OFP)	*C. malherbi*	North Canterbury region, South Island of New Zealand
Reischek's parakeet (ReRCP)	*C. erythrotis hochstetteri*	Antipodes Island, New Zealand
Macquarie Island red-crowned parakeet (MqRCP)	*C. erythrotis*	Formerly on Macquarie Island, Australia (EXTINCT)
Yellow-crowned parakeet (YCP)	*C. auriceps*	North and South Islands of New Zealand, Stewart and Auckland Islands, many other near and offshore Islands of New Zealand
Antipodes Island (green) parakeet (AGP)	*C. unicolor*	Antipodes Island, New Zealand
Norfolk Island red-crowned parakeet (NrRCP)	*C. cooki*	Norfolk Island, Australia
Forbes' parakeet (FP)	*C. forbesi*	Mangere and Little Mangere Islands, Chatham Islands, New Zealand
New Caledonia red-crowned parakeet (NcRCP)	*C. saisetti*	New Caledonia
Lord Howe Island red-crowned parakeet (LhRCP)	*C. n. subflavescens*	Formerly on Lord Howe Island, Australia (EXTINCT)
Kermadec parakeet (KrRCP)	*C. n. cyanurus*	Kermadec Islands, New Zealand
Black-fronted parakeet (BFP)	*C. zelandicus*	Formerly on Tahiti, Society Islands (EXTINCT)
Society parakeet (SP)	*C. ulietanus*	Formerly on Raiatea, Society Islands (EXTINCT)

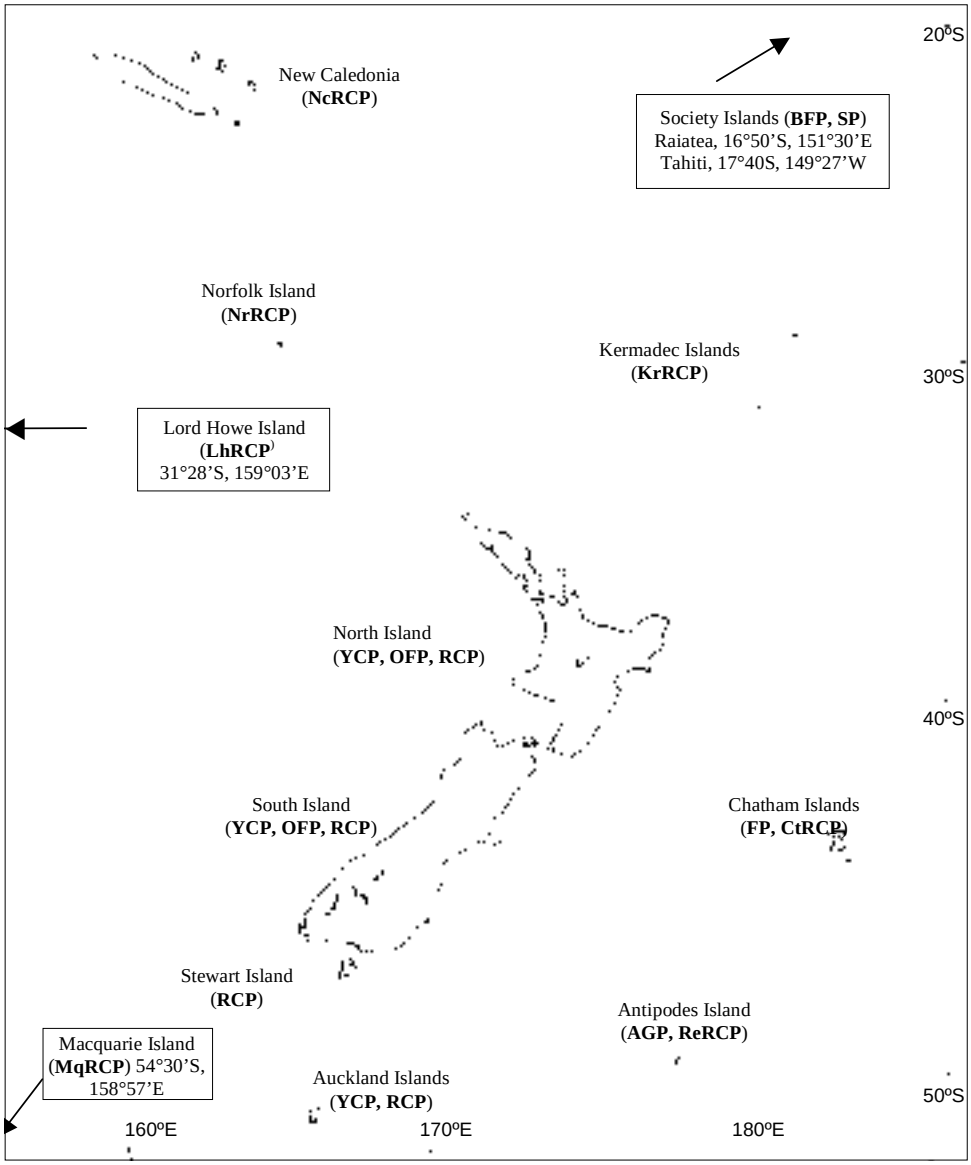
* Species names follow Triggs & Daugherty (1996) and the conclusions of the present report.

(*C. malherbi*) exist in the Hurunui and Hawdon Valleys, North Canterbury, New Zealand. The yellow-crowned parakeet (*C. auriceps*) is the most common taxon, distributed across the larger forest tracts of the North and South Islands and many offshore islands of New Zealand (Taylor 1985). Forbes' parakeet (*C. forbesi*) is limited to Mangere and Little Mangere Islands in the Chathams Group. The *Cyanoramphus* parakeets have adapted to a remarkably wide range of habitats from sub-antarctic tussock (*C. unicolor*, Antipodes Island) to tropical rainforests (*C. saisetti*, New Caledonia).

2.2 TAXONOMY

Most taxonomic work on these species was performed prior to 1900. The taxonomy and classification of *Cyanoramphus* parakeets was largely based on external morphology and biogeography without explicit reference to species concepts (e.g. Oliver 1930) or genetic data. As a consequence, the systematics

Figure 1. Distribution of *Cyanoramphus* parakeets.



Notes:
AGP, Antipodes Island (green) parakeet *C. unicolor*;
BFP, black-fronted parakeet *C. zelandicus* (extinct);
CtRCP, Chatham Island red-crowned parakeet *C. n. chathamensis*;
FP, Forbes' parakeet *C. forbesi*;
KrRCP, Kermadec parakeet *C. n. cyanurus*;
LhRCP, Lord Howe Island red-crowned parakeet *C. n. subflavescens* (extinct);
MqRCP, Macquarie Island red-crowned parakeet *C. erythrotis* (extinct);
NcRCP, New Caledonia red-crowned parakeet *C. saisetti*;
NrRCP, Norfolk Island red-crowned parakeet *C. cooki*;
OFP, orange-fronted parakeet *C. malherbi*;
RCP, New Zealand red-crowned parakeet *C. novaezelandiae*;
ReRCP, Reischek's parakeet *C. e. hochstetteri*;
SP, Society parakeet *C. ulietanus* (extinct);
YCP, yellow-crowned parakeet *C. auriceps*.

of *Cyanoramphus* and the species' names have changed frequently (see Boon et al. 2000a & in press). Based on classical taxonomy, the final estimate (Forshaw & Cooper 1989) of the number of *Cyanoramphus* species stood at five with a further nine subspecies.

Management of genetic diversity requires correct identification of species, the relationships between species and a thorough knowledge of the geographic structure of populations (Triggs & Daugherty 1996). Failure to recognise existing diversity may deny some species proper conservation protection and condemn them to extinction (Daugherty et al. 1990). The preliminary analysis conducted by Evans (1994) was unsuccessful in delineating *Cyanoramphus* taxa at the species and subspecies level due to inadequate resolution afforded by DNA sequence data from the mitochondrial 12S rRNA gene locus. A later report on genetic variation at 21 allozyme loci allowed Triggs & Daugherty (1996) to propose that Forbes' (*C. forbesi*) and orange-fronted parakeets (*C. malherbi*) warranted full species status. They also concluded that the sister taxon of Forbes' parakeet was the red-crowned parakeet (*C. novaezelandiae*) rather than the yellow-crowned parakeet (*C. auriceps*).

Modern conservation practices are almost synonymous with 'gene pool conservation' (Avise 1989; Brooks et al. 1992; Rojas 1992; Baverstock et al. 1993; Milligan et al. 1994; Hazevoet 1996; Rhymer & Simberloff 1996). Thus, using DNA-based genetic techniques, we have been able to conduct a fine-scale survey of *Cyanoramphus* gene pools. We examined a total of 74 individuals representing most *Cyanoramphus* populations and taxa (except for *C. n. subflavescens, C. n. cyanurus, C. zelandicus and C. ulietanus*) using highly discriminating mitochondrial control region DNA sequence data to explore relationships among the species and subspecies belonging to this parakeet complex. We have applied the Phylogenetic Species Concept (Cracraft 1983) to our taxonomic classifications for species, but recognition of subspecies is based on a conservative approach, where they are classified as such if, and only if, they are (or were) morphologically similar to the parent species but form a clearly separate allopatric but closely related clade to the main species.

2.3 MOLECULAR SYSTEMATICS

Molecular techniques provide scientists with the ability to analyse organisms non-invasively (an important attribute when dealing with endangered species) and provide the independent and relatively objective estimates of phylogeny needed to infer evolutionary histories and mechanisms of speciation. Mitochondrial DNA (mtDNA) analysis is a powerful tool for evolutionary studies and can provide insights into population structure, gene flow, hybridisation, biogeography and phylogenetic relationships (Moritz et al. 1987). Many qualities of mtDNA make it an ideal genetic marker for evolutionary studies. Some of its more useful features include high copy number (therefore ease of purification and amplification), a generally observed maternal mode of inheritance (clonally inherited from the female parent to the offspring without recombination in most cases) and accelerated mutation rate relative to the nuclear genome (providing a sensitive monitor over short time scales). Varying functional constraints on

different parts of the mitochondrial genome means that mtDNA genes evolve at different rates. These provide genes or target regions within this genome that are capable of yielding data to answer evolutionary and speciation questions at different temporal or taxonomic scales (i.e. deep versus shallow divergences). The flexibility afforded by mtDNA sequence analysis facilitates testing of some highly complex evolutionary models (Cooper et al. 1992; Hedges 1994; Baker et al. 1995; Cooper & Penny 1997; Hedges & Poling 1999).

2.4 BIOGEOGRAPHY, SPECIATION AND EVOLUTION

Speciation is a core process in evolution. It is often correlated with the separation of a gene pool into identifiable units driven by genetic drift due to vicariant or founder events or by sexual or disruptive natural selection. This may occur in allopatry or sympatry (see Templeton 1981 and Tregenza & Butlin 1999 for interesting discussions on the mechanisms and nature of speciation). The fixation of a particular genotype signals the emergence of a new species through ecological isolation (pre-zygotic) and, ultimately, physiological (post-zygotic) isolation. The latter phenomenon is rarely observed at an early stage of the speciation process in the class Aves. Most avian speciations are a result of pre-zygotic isolation, where differences in plumage and courtship patterns are a focus for disruptive sexual selection (see Grant & Grant 1992, 1997).

Cyanoramphus parakeets are a good example of this mode of speciation. Conclusions based on our genetic survey allow us to address issues on speciation in the genus and also to explore the possible origins of these birds. Recent geological evidence suggests the existence of a discontinuous land bridge between New Caledonia and New Zealand (late Paleogene to early Neogene; Herzer et al. 1997) which could have permitted unidirectional transfer of species from New Caledonia to New Zealand. We have combined our inferred phylogeny with this new geological theory and other alternative hypotheses in an attempt to explain the origins of New Zealand *Cyanoramphus* parakeets.

3. Objectives

This study aims to provide a revised, comprehensive overview of Kakariki with respect to their taxonomy, relationships between them and their evolutionary history. The main objectives are:

1. To identify and design a molecular analytical system for *Cyanoramphus* parakeets suitable for a fine-scale genetic survey of all populations and taxa belonging to this genus.
2. To produce a detailed phylogeny of *Cyanoramphus* parakeets using data obtained by application of the analytical system described above.
3. To make recommendations for any required changes to the taxonomic ranks of currently recognised species and subspecies based on the new molecular

phylogeny with particular reference to the status of the orange-fronted parakeet (*C. malherbi*).

4. To use the inferred phylogeny to identify fundamental 'Management Units' (Moritz 1994) for conservation of this complex of parakeets.
5. To use the results to estimate species divergence times, infer evolutionary history and suggest a possible speciation pathway for the Kakariki.
6. To use this information to test the evolutionary relationships among members of the genus inferred by Triggs & Daugherty (1996).

4. Materials and methods

4.1 MATERIALS

One of blood, feather, muscle or liver tissues was collected from 90 *Cyanoramphus* parakeets. Only 74 samples produced DNA sequences of high enough quality for phylogenetic analyses. They included New Zealand red-crowned, yellow-crowned, orange-fronted, Antipodes Island (green), New Caledonian red-crowned, Reischek's, Macquarie Island red-crowned, Chatham red-crowned, Norfolk Island red-crowned and Forbes' Parakeets. A detailed list of these samples is given in Appendix 1. They are now stored permanently at –80°C at the Institute of Molecular Systematics, Victoria University of Wellington as part of the National Frozen Tissue Collection of New Zealand. Complete mitochondrial control region DNA sequences were collected (see Section 4.3) from each individual (except for specimen AV2099) for phylogenetic analyses.

4.2 ANALYTICAL SYSTEM DEVELOPMENT

Initial genetic surveys conducted were on the mtDNA cytochrome *b* gene. Polymerase Chain Reaction (PCR) amplifications were performed on this gene using primer pair L14987 (modified from primer 14841, Kocher et al. 1989) / H15305 (G.K. Chambers, pers comm.) which produced a 317 bp product. The low number of phylogenetically informative characters observed in comparisons between distantly related *Cyanoramphus* taxa (see Boon et al. 2000a & and in press) prompted the development of a new set of primers to allow amplification and sequencing of the entire cytochrome *b* gene. Using primer pairs H16065 (modified from primer 15915, Irwin et al. 1991) / L15132 (modified from primer CBII, Dawson 1992) and L14827 (Helm-Bychowski & Cracraft 1993) / 15305 (modified from primer 15149, Kocher et al. 1989), two overlapping 477 and 932 nt targets were amplified. By sequencing inwards from the position of the PCR amplification primers, internal primers were then designed based on the sequence obtained to complete the full sequence of both amplified targets and hence of the complete cytochrome *b* gene. Though informative near the generic level, this target was not deemed to be appropriate for robust separation of recently diverged species or for differentiation of their populations.

Following the pilot study using the cytochrome *b* gene, the fastest evolving segment of the mitochondrial genome—the control region—was next chosen for analysis. Initial attempts at using well-established avian control region PCR primers (Quinn & Wilson 1993; Wenink et al. 1994; Quinn & Mindell 1996) failed to produce any amplification product. This was due to poor matching between the sequences of these primers and the *Cyanoramphus* mitochondrial control region. Long PCR (see Nelson et al. 1996, Hecimovic et al. 1997) using primer pair L15132 / H3780 (Table 2) yielded a target product of 5.6 kb, a size which was too large (Fig. 2) to be easily analysed by the chromosome walking technique described earlier for the two cytochrome *b* products. Several unsuccessful attempts were made to clone these 5.6 kb products. When these failed (for a variety of technical reasons) a 12S rRNA primer (H2152) in close proximity to the mitochondrial control region was trialed as an internal sequencing primer for the 5.6 kb target. This method returned DNA sequence from inside the control region of the *Cyanoramphus* mitochondrial genome. This allowed other sequencing primers to be designed within the specified sub-target (i.e. the control region) and ultimately a system for sequencing the complete control region by chromosome walking. In retrospect, sequencing from the long PCR target during the development of the control region analytical system confirmed that cytochrome *b*, 12S rRNA, 16S rRNA, ND6, $tRNA^{Thr}$, $tRNA^{Glu}$, $tRNA^{Phe}$, $tRNA^{Val}$, $tRNA^{Pro}$ and the control region were all present on a contiguous stretch of DNA.

TABLE 2. SYNTHETIC OLIGONUCLEOTIDE PRIMERS USED FOR PCR AMPLIFICATION AND CYCLE SEQUENCING.

NAME	SEQUENCE (5' TO 3')	REFERENCE
L70-90 (a)	GTA CGT CAC GGG CTC TTT TAG TCC	Boon et al. 2000a
L70-90 (b)	GTC ACG GGC TCT TTT AGT CCT TTA TGG	Boon et al. 2000a
L90-110	AAC TTC ACG CCC TCG GAT AGA ATA	Boon et al. 2000a
L531	TGC TCT TTT GTG CCT CTG GTT CCT C	Boon et al. 2000a
L650	AGC GCC TTG TCT CTG TTG G	Boon et al. 2000a
L14827	CCA CAC TCC ACA CAG GCC TAA TTA A	Helm-Bychowski & Cracraft 1993
L14987	CCC CTC AAA TAT CTC CAT ATG ATG	Boon et al. 2000a
L15132	CGA ACC GTA CAA TAC GGA TGG YTA ATC	Boon et al. 2000a
L15643	CTA CCC TAG CCC TCT TCT CAC CCA ACC TAC	Boon et al. 2000a
L16518	GAC GGG AAT AAA CAA AAA CCA CCA ACA	Boon et al. 2000a
H100-200	GAC TGA AGT GAG ACT ATT CCT TGA GAC	Boon et al. 2000a
H519	ATG CGA CTT GAC CGA GGA ACC AGA GG	Boon et al. 2000a
H646	GGC TAC CCA GAG AAA AAA AAC CAA C	Boon et al. 2000a
H1529	TGG CTG GCA CAA GAT TTA CCG	Boon et al. 2000a
H1800	CCC CCG TTT GTG CTC GTA GTT CTC	Boon et al. 2000a
H2152	GAG GGT GAC GGG CGG TGT GT	Kocher et al. 1989, primer H1478 with 5' end modified.
H3780	CTC CGG TCT GAA CTC AGA TCA CGT AGG	Hedges 1994, primer 16H1.
H15163	GGC GAT GTG GAG GTC GAT GCA GAT GAA GAA	Boon et al. 2000a
H15305	AAA CTG CAG CCC CTC AGA ATG ATA TTT	Boon et al. 2000a
H15706	GGC AAA TAG GAA RTA TCA TTC	Boon et al. 2000a
H15977	AGA TGA TGG GGA ATA GGA TTA GGA TGA	Boon et al. 2000a
H16065	TCA TCT CCG GTT TAC AAG AC	Boon et al. 2000a

The letters (L) and (H) refer to the light and heavy strands of the mitochondrial genome and numbers to the 3' nucleotide of the primer relative to the chicken mitochondrial DNA sequence (Desjardins & Morais 1990). Some primers used do not align well with the chicken mitochondrial DNA sequence, thus a range of nucleotide positions are given instead of absolute positions.

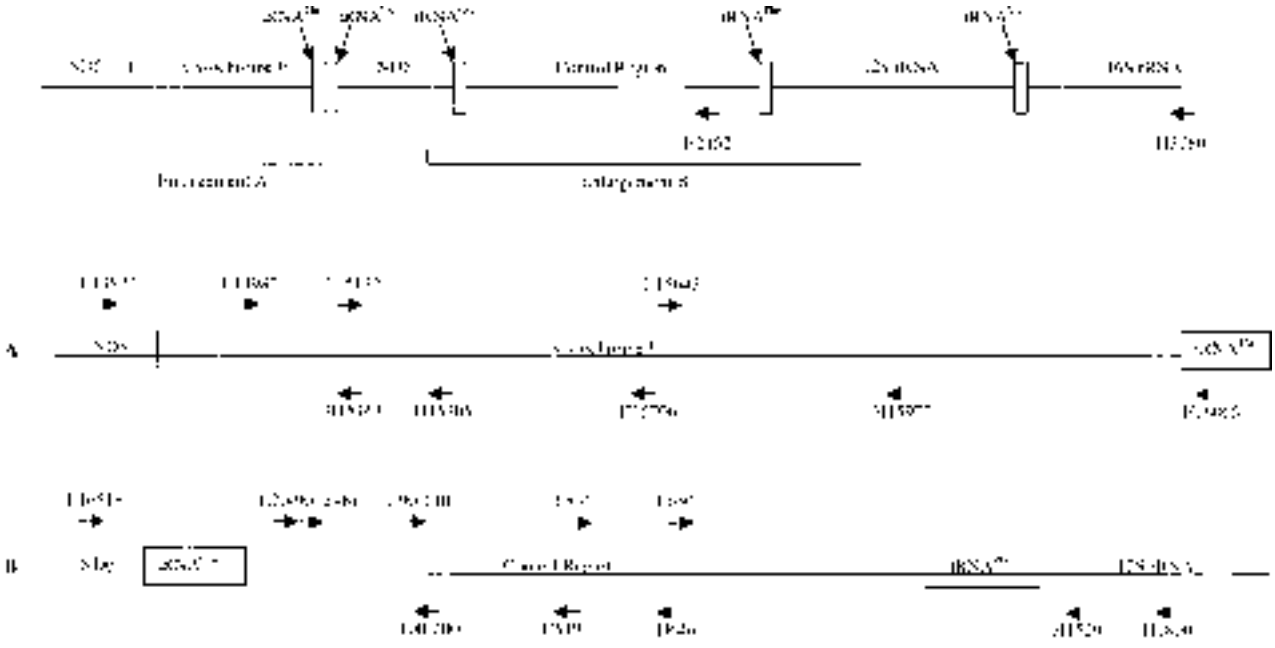


Figure 2. Strategy for PCR amplification and cycle sequencing of the mitochondrial cytochrome *b* and control region.
Note:
Arrows denote primers and their orientations relative to adjacent mitochondrial genetic loci. The alpha numeric designation of primers refer to the 'light (L)' and 'heavy (H)' strands of the mitochondrial DNA plus their positions relative to the chicken mitochondrial DNA sequence (Desjardins & Morais 1990). The sizes of gene targets are not drawn to scale.

4.3 METHODS

4.3.1 DNA extraction, gene target amplification and DNA sequencing

DNA extraction and mitochondrial cytochrome *b* and control region gene target amplifications were performed according to Boon et al. (2000a). DNA extractions of feather tissues was performed using the modified SDS-proteinase K-dithiothreitol digestion of Leeton & Christidis (1993). DNA sequencing was also performed according to Boon et al. (2000a).

4.3.2 Ancient DNA techniques

DNA extraction procedures on feathers obtained from museum skins and PCR amplification of gene targets were performed in a purpose-built, ultraclean 'ancient DNA' laboratory in the Institute for Molecular Systematics in Victoria University of Wellington. A small genetic target of 354 bp was analysed for the museum sample AV2099 using primer pair H100-200 / L70-90(b) because the usual 2.1 or 2.5 kb target was not amplifiable due, we expect, to the to highly fragmented DNA molecules often found in samples obtained from museum skins. Pre- and post-PCR procedures were carried out in separate laboratories, each completely and separately equipped. No DNA work had previously been carried out in the ancient DNA facility. All PCR set-ups were performed in a laminar flow hood and all other procedures were performed while wearing face mask, shower cap and disposable gloves to lower the risk of contamination by human hair and skin particles. Aerosol barrier pipette tips were used at all

times. Ultra high quality water used for all ancient DNA procedures was prepared by double distillation and autoclaving. Following that, the water was sterile microfiltered into 1.0 ml aliquots and UV-C (254 nm) irradiated for two hours. The ancient DNA laboratory itself was UV-C irradiated for 30 minutes before and after experiments were carried out and negative controls for extractions and PCR experiments were included at all times.

4.4 PHYLOGENETIC ANALYSIS

4.4.1 Phylogeny reconstruction

DNA sequences were obtained from both light and heavy strands of the mitochondrial control region and combined to produce unambiguous contiguous sequence files with the DNASTAR Inc. Lasergene data acquisition and analysis package (Anon 1997). Consensus DNA sequences for each individual were aligned with Lasergene's MegAlign program (Anon 1997) using the Clustal-V (Higgins & Sharp 1989) algorithm and exported to PAUP*4.0b2 (Swofford 1998) for phylogenetic analysis.

After the sequences were aligned, the differences between them were estimated by calculating numbers of nucleotide substitutions. In order to accurately estimate genetic distances from substitution numbers, multiple hits (undetected substitutions), transition:transversion (ts:tv) ratio differences, nucleotide composition bias and variations in rate of change between different sites in the dataset needed to be corrected for. We therefore tested which model of sequence evolution (i.e. nucleotide substitution) best fit the overall dataset (see Hillis et al. 1996). We found that the GTR + I + G model was the most appropriate to describe the base substitution process for the 'complete' (73-taxa) and 'reduced' (23-taxa) datasets among all the models examined.

Initially, a heuristic parsimony tree search under equal weights was performed on the complete 73-taxon dataset with a restricted number of 1000 trees allowed to be saved. A neighbour-joining tree was also constructed from the complete (73-taxon dataset) dataset for comparison with the parsimony analysis. Following that, tree searches (23-taxon dataset) under maximum likelihood and minimum evolution optimality criteria were performed based on the GTR + I + G model of base substitution, using the parameters (I = 0.56 and α = 0.57) estimated from the 23-taxon dataset.

Tree searches under maximum likelihood optimality criteria were also performed based on the JC69, K80 and HKY85 with rate heterogeneity (I + G) using the parameters estimated from the 23-taxon dataset to ensure that the model of evolution used for tree building produced consistent results.

Bootstrap resampling was carried out in each analysis in order to provide an assessment of statistical support for each bifurcating node (branch point in the tree). To analyse the affinity of the extinct Macquarie Island red-crowned parakeet (specimen AV2099) from which only 354 bp sequence was considered, a special reduced dataset was aligned with the corresponding region for 31 other *Cyanoramphus* sequences which included all red-crowned parakeets and one representative from each other species.

4.4.2 TESTING THE MOLECULAR CLOCK

Only one taxon from each of the main clades revealed in previous analyses was chosen for this exercise due to the computational burden involved in phylogenetic analyses with a molecular clock enforced. The total number of sequences used for this analysis was twelve. Initially, an alignment of the full mitochondrial control region sequences was made from 12 selected *Cyanoramphus* taxa, chicken (Desjardins & Morais 1990), guillemot (Kidd & Friesen 1998), dunlin and turnstone (Wenink et al. 1994). Later, sections of the control region were assigned as domain (D) one, two and three (Baker & Marshall 1997). The central conserved domain corresponded to positions 784 to 980 of the 1613 aligned sites in the 12-taxon dataset. Domains one and three corresponded to positions 1 to 783 and 981 to 1613 respectively. It has previously been demonstrated (Saccone et al. 1991) that only the central conserved domain 2 of this locus behaves as a reliable molecular clock. However, for completeness, the combination of domains 1 + 2, 1 + 3, 2 + 3 and 1 + 2 + 3 were tested for molecular clock conformity as were individual domains 1, 2 and 3. The substitution model fitting process was performed independently on each combination of domains and appropriate models selected for use in maximum likelihood tree inference according to the criteria.

To search for a reliable 'molecular clock' in our dataset, a heuristic search was performed for each of the domain combinations and the likelihood score of each resulting tree recorded. The likelihood score (under molecular clock assumption) was then estimated from the shortest tree found previously and rooting it using the New Caledonian red-crowned parakeet taxon (RCP00129) and the 'tree scores' option in PAUP*4.0b2 (Swofford 1998). Likelihood ratio tests (see Sorhannus & Van Bell 1999) were employed which utilize the likelihood scores found for each dataset with and without the molecular clock assumption. It was then found that D2 + 3 behaves in a clock-like manner, thus allowing a time-constrained tree (i.e. branch lengths are directly proportional to time of divergence) to be constructed. To add a temporal scale to the time-constrained tree, the amount of genetic divergence per unit of time needs to be established. Prior to rate estimations, a matrix of maximum likelihood rates was computed for the dataset (domains 2 + 3) under the HKY85 + G model of substitution to correct for ts:tv ratio differences, base composition bias and among-site rate variation. The rate of sequence change was estimated for domains 2 + 3 by calculating the relative-rate ratio between the substitution rate matrices of domain 1 and domains 2 + 3. An average of the relative rate values based on twelve *Cyanoramphus* taxa was calculated to produce a conversion factor with which to estimate the relative rate of evolution for domains 2 + 3 combined. The rate of evolution was calculated based on a rate of 20.8% per Myr for domain 1 of the control region as proposed by Quinn (1992, see Marshall & Baker 1998 for an alternative view). A simpler version of this type of procedure was used in Wenink et al. (1996) on a Dunlin (*Calidris alpina)* dataset.

To support our rate estimates from the mitochondrial control region, we also utilised the cytochrome *b* data from Boon et al. 2000a and in press and Leeton et al. (1994) to produce independent time point calibration. Here we used transversion substitutions only to estimate divergence times from the cytochrome *b* data. The rate of transversion substitutions for our dataset was

0.20% per Myr (Irwin et al. 1991). Finally, the branch lengths of the time-constrained maximum likelihood phylogram were used to estimate the timing for evolutionary events in the *Cyanoramphus* phylogeny. This provides us with a temporal scale that can be used to search for correlation between geological and evolutionary events.

5. Results

5.1 DNA EXTRACTION

All samples collected from 1996 onwards yielded high quality DNA. Partial degradation of DNA was observed in other samples analysed in this study, but they proved useable as templates for PCR amplifications, after optimisation of procedures involving alterations to thermal cycling reagents and conditions. The specimens listed in Appendix 1 underwent DNA extraction procedures.

5.2 MITOCHONDRIAL CONTROL REGION

The complete control region DNA sequences from the specimens analysed vary in length from 1558 to 1601 nucleotides due to insertions and deletions, a feature characteristic of this hypervariable region of the mitochondrial genome. When all 73 taxa were aligned using the clustal algorithm, a total of 1620 sites were considered for phylogenetic analyses (full alignments can be obtained from WMB). Optimal alignment for the 'reduced' 23-taxon dataset produced an alignment with only 1616 sites.

In the large 73-taxon dataset, a total of 329 variable sites were identified. They provided sufficient characters for cladistic-, distance- and likelihood-based methods of analyses. The highest level of intra-specific variation was observed within the New Zealand red-crowned parakeet species, which ranged between 0.00% and 3.11%. The corresponding levels of intra-specific divergence were 0.00–0.12% for the Antipodes Island (green) parakeet, 0.00–1.79% for the New Caledonian red-crowned parakeet, 0.00–0.12% for the Norfolk Island red-crowned parakeet, 0.00–7.65% for Forbes' parakeet (HG1 - 3, see Fig. 3), 0.00–1.46% for the yellow-crowned parakeet and 0.00–0.82% for the orange-fronted parakeet. The uncorrected percentage difference for inter-specific comparisons ranged from 0.95% to 9.82%. The maximum likelihood estimate of transition:transversion (ts:tv) ratios ranged from 5.01 to 11.27 but when a larger number of sequences were used for estimation, the ts:tv ratio tended towards the lower bound of 5.01.

5.3 PHYLOGENETIC ANALYSIS

A total of 271 parsimony informative characters were available for analysis of the full 73-taxon dataset. Parsimony analysis separates the proposed species into well supported monophyletic clades with bootstrap values ranging from

Figure 3. Neighbour-joining phylogeny of 73 *Cyanoramphus* individuals based on analysis of 1620 aligned mitochondrial control region sites.

Note:

The General-Time-Reversible model of substitution (Rodriguez et al. 1990; Yang 1994) with rate heterogeneity were used for analysis (I = 0.56, α = 0.57). Bootstrap values greater than 80% supporting haplotypes and taxonomic subdivisions are indicated at respective nodes (1000 replicates). Each individual is labelled based on sampling location and codes given in parentheses refer to their individual collection numbers as part of the New Zealand National Frozen Tissue collection.

See Fig. 1 for abbreviations.

71% to 100% in trees of 580 steps (tree not shown). The neighbour-joining phylogeny (Fig. 3) is almost identical to the maximum parsimony, differing only with respect to detailed relationships at the terminal 'twigs' of the trees. One tree was recovered from the minimum evolution analysis revealing a phylogenetic pattern which was broadly congruent with that obtained under maximum parsimony (trees not shown). Bootstrap values ranged between 57% and 100% for the main clades in the minimum evolution analysis. Two equally likely trees were found (*-ln* likelihood = 4539.24) in the maximum likelihood heuristic search (trees not shown). Both trees had topologies comparable with those obtained using maximum parsimony and distance methods.

The inferred phylogenetic pattern from the 'reduced' 23-taxon dataset did not appear to be greatly affected by the use of different models of substitutions. However, as more general models were applied, the bootstrap values tended to decrease. The only exception was the use of the HKY85 model. The inclusion of among-site rate variation influences the likelihood scores of the inferred phylogenetic trees to a large extent. This phenomenon was observed irrespective of the types of parameters incorporated into the phylogenetic inference. Likelihood scores varied considerably when gamma approximations (G) were not included in the analyses, but the inclusion of invariable sites (I) calculations did not affect the likelihood values greatly.

The main clades (Fig. 3) consist of the Chatham Island red-crowned parakeet (*C. n. chathamensis*), New Zealand red-crowned parakeet (*C. novaezelandiae*), orange-fronted parakeet (*C. malherbi*), Reischek's parakeet (*C. e. hochstetteri*), yellow-crowned parakeet (*C. auriceps*), Antipodes Island (green) parakeet (*C. unicolor*), Norfolk Island red-crowned parakeet (*C. cooki*), Forbes' parakeet (*C. forbesi*) and New Caledonian red-crowned parakeet (*C. saisetti*).

Interestingly, Forbes' parakeet samples returned three main DNA sequence haplotype groups (see also Boon et al. 2000b). The first (HG1: CD1816, CD1821) was embedded within the New Zealand red-crowned parakeet clade, the second (HG2: CD1811, CD1818, PMC26) was basal to the New Zealand red-crowned parakeet clade and the third (HG3: CD1814, CD1815, CD1817, CD1810, CD1820, CD1819) was basal to all other New Zealand *Cyanoramphus* parakeets (see Fig. 3).

Three possible haplotype groups (clades) of the New Zealand red-crowned parakeet were apparent (see Fig. 3, nodes represented by bootstrap values of 90%, 94%, 95% & 99% within the New Zealand red-crowned parakeet clade). These genetic partitions are not correlated to geographical separations in any discernable way. The Chatham Island red-crowned parakeet forms a distinct group within the red-crowned parakeets, supporting its current subspecific classification. The Reischek's parakeet sequences group together in a monophyletic clade on their own, which is diagnosable from the New Zealand red-crowned parakeet clade. The extinct Macquarie Island red-crowned parakeet (*C. erythrotis*, see discussion) is the sister taxon of Reischek's parakeet (Fig. 4). The latter species had two distinct haplotypes, one consisting of individuals RE1, RE2, RE3, CD968, CD969 and the other with RE4 alone.

The orange-fronted parakeet forms a monophyletic clade on its own within the main red-crowned parakeet clade (Fig. 3). Three haplotypes, one represented by a single Hope Valley bird (JK1) and two by the remaining nine individuals

Figure 4. Strict consensus of 48 equally parsimonious trees (481 steps) based on 1616 aligned mitochondrial control region sites of all available red-crowned parakeet data and one representative from each species and subspecies. The resulting number of taxa is 32. The extinct Macquarie Island red-crowned parakeet position in the *Cyanoramphus* phylogeny is highlighted with a box. Codes given in parentheses refer to their individual collection numbers as part of the New Zealand National Frozen Tissue collection.
See Fig. 1 for abbreviations.

from Hurunui Valley, were apparent within the orange-fronted parakeet clade. A single yellow-crowned parakeet individual (FT1018) appears as basal taxon to the orange-fronted parakeet clade, while two morphologically orange-fronted Hope Valley birds (IS1 and WG612) were included in the yellow-crowned parakeet complex (Fig. 3). These demonstrate the possible occurrence of birds that may be genetically different from their apparent morphology. The yellow-crowned parakeet clade showed low levels of population structuring with perhaps five haplotype groups within the complex. These groups are represented by nodes with bootstrap support of more than 50% within the yellow-crowned parakeet clade (Fig. 3). Yellow-crowned parakeets from the South and North Islands appeared to be separated into different haplotype groups, although two of the specimens examined (FT3308—Hawdon Valley and PSF—Pureora Forest) were not resolved into any specific haplotype group.

The New Caledonian red-crowned parakeet sequences are basal to all extant *Cyanoramphus* examined in this study, followed by grouping of Forbes' parakeet (haplotype group 3), the Norfolk Island red-crowned parakeet and Antipodes Island (green) parakeet. The latter two species alternate in their relative positions in various analyses and have low bootstrap support due to the short internode separating these two clades.

5.3.1 MOLECULAR CLOCK TEST AND DATE ESTIMATES

We have estimated an evolutionary rate of 6.80% per Myr for the combined dataset of domains 2 + 3 of the *Cyanoramphus* mitochondrial control region. Using only transversion data from the cytochrome *b* locus (see Boon et al. 2000a), we have estimated that the Australian *Platycercus* spp. would have been separated from New Zealand *Cyanoramphus* spp. for at least 20 Myr (see Appendix 2). Pairwise comparisons between the New Caledonian taxa *Eunymphicus* spp. and the *Platycercus* spp. show levels of genetic divergence similar to comparisons between *Cyanoramphus* spp. and *Platycercus* spp. although, generally, the latter comparisons show marginally higher levels of divergence. The *Eunymphicus* spp. shows genetic affinity to the New Caledonian endemic *Cyanoramphus* species (*C. saisetti*) with a calculated divergence time of 2.3 Myr (see Appendix 2). Hence the *Eunymphicus*-New Zealand *Cyanoramphus* spp. divergence stands at an estimate of approximately 2.9 Myr while the *C. saisetti*-New Zealand *Cyanoramphus* divergence probably took place within the last 450 000–600 000 years (See Appendix 2). The corresponding time estimate for the latter divergence based on the corrected (see methods) control region data was 625 000 years. The New Zealand radiation of *Cyanoramphus* spp. probably occurred within the last 450 000–625 000 years which would include the Holocene and parts of the Pleistocene (see Fig. 5).

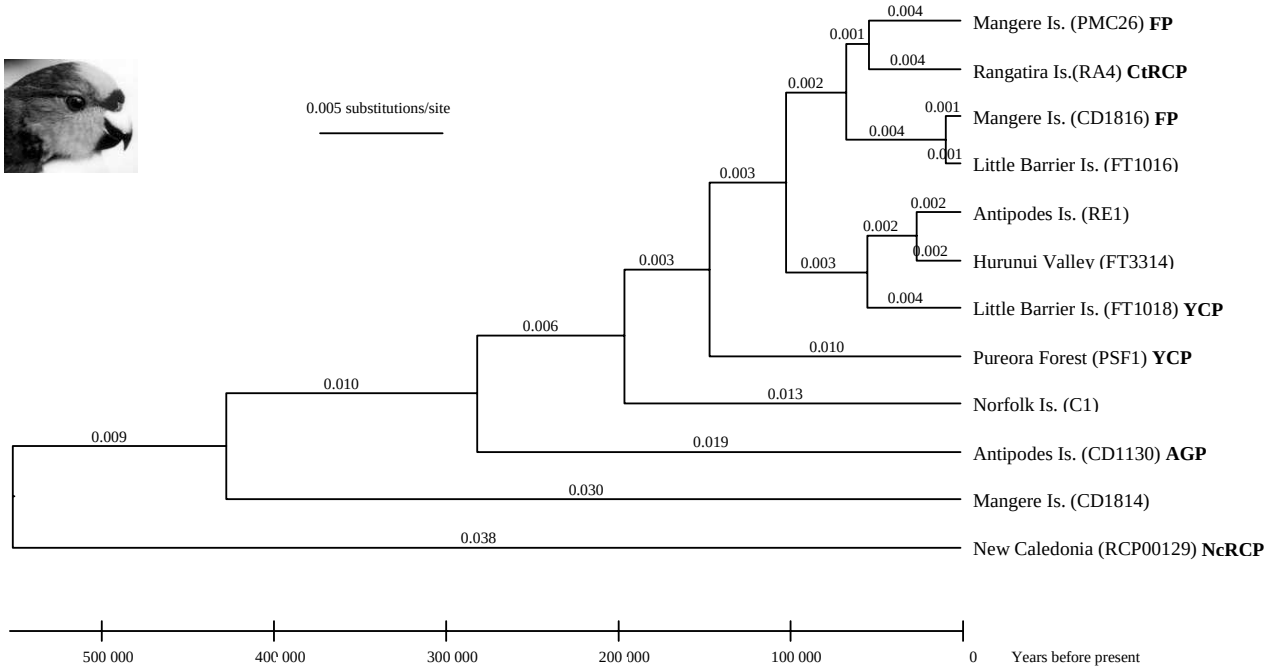


Figure 5. Time-constrained phylogenetic tree for *Cyanoramphus* obtained by maximum likelihood analysis of domains 2 + 3 of the mitochondrial control region. The Hasegawa-Kishino-Yano model of substitution (Hasegawa et al. 1985) with rate heterogeneity were used for analysis (α = 0.14). The numbers above each branch represent the branch lengths measured in substitutions per site. The inferred time scale for evolutionary divergence is indicated above. See Fig. 1 for abbreviations.

6. Discussion

6.1 ANALYTICAL SYSTEM

New sets of oligonucleotide primers have been designed for analysis of the mitochondrial cytochrome *b* gene and the control region in *Cyanoramphus* parakeets. The cytochrome *b* locus was found to be appropriate for analysis at the generic level and the control region was appropriate for specific, subspecific and population levels. This report concentrates on the DNA sequence data of the mitochondrial control region as a tool for inferring a detailed phylogeny of the genus *Cyanoramphus*. All primers were designed to be as flexible as possible, without compromising their specificity for *Cyanoramphus* mitochondrial genome. This strategy was essential to the successful use of these primers across the full range of species and ensures good quality PCR amplification products and sequences.

6.2 MITOCHONDRIAL DNA SEQUENCE AUTHENTICATION

One of the most important procedures in molecular genetic studies is data authentication. It is a necessary precaution because a phylogeny which is inferred from molecular data is only as good as the data on which it is based. In particular, researchers performing PCR-based studies of mitochondrial DNA targets need to be aware of the possibility that nuclear pseudogene copies of the mitochondrial genome can inadvertently be amplified (Zhang & Hewitt 1996; Quinn 1997; MacAvoy & Chambers 1999). Nuclear pseudogenes are mitochondrial genes which have been translocated to the nuclear genome and thus evolves at a much reduced rate with respect to the rate of mitochondrial evolution.

Procedures performed at the stage of 'analytical system development' (Section 4.2) provided opportunities to authenticate the sequences obtained. DNA sequences obtained from a large (5.6 kb) segment of the mitochondrial genome allowed us to confirm that there is a high probability that our sequences were not nuclear homologues of the mitochondrial genome. The presence of molecular signatures characteristic of mitochondrial DNA, such as base composition bias, conserved sequence motifs like CSB-1, C box and D box (see Saccone et al. 1991; Nass 1995; Baker & Marshall 1997) also confirm DNA sequence authenticity as mitochondrial.

Authentication of our cytochrome *b* data used for molecular dating is discussed in Boon et al. (2000a). The use of *Cyanoramphus*-specific primers rather than universal avian primers also reduces the likelihood of the primers amplifying nuclear copies of the mtDNA genome (see Arctander 1995; Sorenson & Fleischer 1996). Overall, we believe that we have taken all reasonable precautions to ensure that all DNA sequences we present here are of genuine mitochondrial origin and, consequently, our phylogenetic analyses are properly based on orthologous genes as opposed to paralogous genes.

6.3 TAXONOMY AND PHYLOGENY OF KAKARIKI

The phylogenetic patterns observed (Fig. 3) suggest that taxonomic practice for this group should be based on the Phylogenetic Species Concept (Cracraft 1983). This states that a species is 'the smallest population or group of populations within which there is a parental pattern of ancestry and descent and which is diagnosable by unique combinations of character states' (Eldredge & Cracraft 1980; Nelson & Platnick 1981; Cracraft 1983; Nixon & Wheeler 1990). The phrase 'parental pattern of ancestry and descent' implies reproductive cohesion over time; the element of 'diagnosability' is specifically mentioned; and the statement referring to the 'smallest population' establishes the basis for ranking. That is, the boundary to species limits is the smallest population or group of populations that is diagnosably distinct (Cracraft 1997). Phylogenetic monophyly implies strict genetic cohesion of a population over time and also establishes the ancestor-descendant relationships of taxa. The genetic distinctiveness of a population thus meets the 'diagnosability' criterion of the Phylogenetic Species Concept. We note that three elements of the Phylogenetic Species Concept-diagnosability, genetic cohesion and monophyly, are not mutually exclusive but complementary.

6.3.1 Red-crowned parakeets

Traditionally, all *Cyanoramphus* parakeets which were predominantly green with a red crown colour have been considered conspecific (*C. novaezelandiae*). They were divided into subspecies based on their allopatric distributions and variation in size and appearance. The genetic data from our samples and analyses support separating the red-crowned parakeet into two monotypic species (*C. cooki* from Norfolk Island, *C. saisetti* from New Caledonia) and two polytypic species (*C. n. novaezelandiae* from mainland New Zealand and *C. n. chathamensis* from Chatham Islands; *C. e. erythrotis* from Macquarie Island (extinct) and *C. e. hochstetteri* from Antipodes Islands). *C. cooki* was previously classified *as C. n. cooki; C. saisetti* as *C. n. saisetti; C. e. erythrotis* as *C. n. erythrotis* and *C. e. hochstetteri* as *C. n. hochstetteri* (Forshaw & Cooper 1989).

The red-crowned parakeets from New Caledonia and Norfolk Island show genetic divergences comparable with other *Cyanoramphus* congeneric species which form monophyletic clades on their own (e.g. Antipodes Island green parakeet, *C. unicolor*). The New Caledonian and Norfolk Island red-crowned parakeets also form monophyletic clades, thus fulfilling the criterion for specific status under the Phylogenetic Species Concept (see Boon et al. in press). We do not have red-crowned parakeet specimens from the Kermadec Islands (presently designated as *C. n. cyanurus*) or from the extinct Lord Howe Island red-crowned parakeet (*C. n. subflavescens*). We cannot, therefore, comment on their phylogenetic status and positions relative to the other taxa we have examined.

Genetic analysis (Fig. 4) of the extinct Macquarie Island red-crowned parakeet (*C. erythrotis*) shows its close affinity to Reischek's parakeet (*C. e. hochstetteri*) which suggests that Macquarie Island may have been colonised by Reischek's parakeet in the past or (vice versa). The nomenclature for Reischek's parakeet and Macquarie Island red-crowned parakeet should be reversed allowing the Macquarie Island red-crowned parakeet to be elevated to species status as *C. erythrotis* and Reischek's parakeet to be a subspecies of the Macquarie bird, as *C. e. hochstetteri*. This is based on conventional taxonomic rules whereby the earlier species name ('*erythrotis*', Wagler 1832—cited in Higgins 1999) should be used as the specific designation and the later species name ('*hochstetteri*', Reischek 1889—cited in Higgins 1999) should be the subspecific designation.

Nevertheless, due to the relatively close proximity of the Antipodes Island to New Zealand, it is more likely that Macquarie Island was colonised from the Antipodes Island after *Cyanoramphus* had become established in the Antipodes Island. The monophyly and diagnosability of the Macquarie Island red-crowned parakeet and Reischek's parakeet (Figs 3 & 4) sequences allow the elevation of the former taxon to species level as *C. erythrotis* and the nomenclature for the latter be changed from *C. n. hochstetteri* to *C. e. hochstetteri*. It is important to note, however, that this result is based on only one sample of the Macquarie Island red-crowned parakeet because only one museum skin of this extinct species was available for analysis.

The phylogenetic position of Chatham Island red-crowned parakeets within the red-crowned parakeet clade fully justifies retention of their status as subspecies—*C. n. chathamensis* of the New Zealand taxon *C. novaezelandiae*.

This decision is supported by its allopatric distribution, its morphological similarity to and its genetically distinct lineage from the New Zealand mainland red-crowned parakeet. The same criteria apply to the Macquarie Island red-crowned parakeet relative to the Antipodes Island Reischek's Parakeet (or vice-versa). In all respects, the Chatham Island red-crowned parakeet should be viewed as a 'Management Unit' (*sensu* Moritz 1994) and, as such, accorded an appropriately significant conservation status.

6.3.2 Orange-fronted parakeet

The orange-fronted parakeet represents a species in its own right based on the genetic data we have presented here and previously (see Boon et al. 2000a for in-depth discussion). It was previously classified as a colour morph of the more common yellow-crowned parakeet (Taylor et al. 1986). First, they form a diagnosable, monophyletic assemblage (Fig. 3) which is sister to the Reischek's parakeet clade and not to the yellow-crowned parakeet (*C. auriceps*). Second, high genetic divergence of the orange-fronted parakeet from both red and yellow-crowned parakeets is observed (Fig. 3). Third, pre-mating isolation of the orange-fronted parakeet from the sympatric population of yellow-crowned parakeet supports strongly its status as a full species—*C. malherbi* (see Boon et al. 2000a). The two mtDNA haplotypes observed within the orange-fronted parakeet clade may not reflect any real form of geographically correlated genetic separation due to the close proximity of Hope and Hurunui Valleys. Birds in this region may well share the same gene pool, as shown by the occurrence of Hope Valley birds with the Hurunui Valley haplotype. If a larger number of Hope Valley specimens were to be analysed, we might find that birds from both valleys share haplotypes.

Our data show that the orange-fronted parakeet shared a more recent common ancestor with red-crowned parakeets as opposed to the yellow-crowned parakeet. The sister taxon of the orange-fronted parakeet is Reischek's parakeet (Antipodes Island red-crowned parakeet, *C. e. hochstetteri*). This is the only red-crowned parakeet with orange-red markings on its head and on each side of its rump, which may support further their close genetic proximity (Forshaw and Cooper 1989).

The New Zealand red-crowned parakeet (*C. novaezelandiae*) was known to occur throughout the North Island, South Island, Stewart Island and Auckland Islands (Oliver 1955), although they do not currently exist in the North Canterbury region where orange-fronted parakeets are found. However, orange-fronted parakeets were once found in the North and South Islands of New Zealand, and the two species may have been sympatric in some parts of their ranges (Buller 1869, 1882, 1884; Reischek 1887, 1952; Taylor 1998). The orange-fronted parakeet population has declined in the last two decades, contributing to the lack of conspecific mates for the orange-fronted parakeet in the Hope Valley. Two of the Hope Valley orange-fronted parakeet individuals (IS1 & WG612) have haplotypes that resemble that of the Eglinton Valley yellow-crowned parakeet (Fig. 3). We interpret them as being the result of previous inter-specific hybridisations in the Hope Valley. This phenomenon has occurred in the Auckland Islands (yellow x New Zealand red-crowned parakeets) and on Mangere Island (Forbes' x Chatham Island red-crowned parakeets). To these, we now add the Hope Valley (orange x yellow-crowned parakeets) as shown by our genetic data.

6.3.3 Yellow-crowned parakeet

The yellow-crowned parakeet forms a clade with low levels of intra-specific genetic variation, thus supporting its specific status as *C. auriceps*. From the data presented here, it is premature to conclude that there is a distinct genetic separation of the North and South Island yellow-crowned parakeets, because some of the terminal taxa within the yellow-crowned parakeet clade were not resolved into any particular haplotype group (Fig. 3). We cannot, therefore, corroborate Triggs & Daugherty 1996. Nevertheless, a study with higher genetic resolution and larger sample sizes may provide alternative evidence.

Individual FT1018 (morphologically recognised as a yellow-crowned parakeet) from Little Barrier Island is particularly interesting as it may represent the North Island haplotype of the now extinct North Island orange-fronted parakeet population. The occurrence of the orange-fronted parakeet in the North Island of New Zealand is perhaps a debatable issue (Higgins 1999), but we have been able to obtain orange-fronted parakeet specimens labelled with North Island place names from the Australian Museum, Sydney (collected in Drury) and the Vienna Museum (collected from Taranga Island) for genetic analysis. None of these have yet yielded DNA sequences, and thus the genetic identity of the North Island orange-fronted parakeet remains to be identified.

6.3.4 Antipodes Island (green) parakeet

The Antipodes Island (green) parakeet has always been classified as a full species assumed to be basal to all other New Zealand parakeet taxa (this study; Triggs & Daugherty 1996; Boon et al. 2000a and in press). Its placement change relative to Forbes' parakeet is, however, a novel finding, and the phylogeny of Forbes' parakeet is discussed further below. The deep separation of the Antipodes Island (green) parakeet from other New Zealand taxa shown in our data suggests that the colonisation of Antipodes Island by *Cyanoramphus* must have occurred prior to the radiation of the red, yellow and orange-fronted parakeets (excluding the Norfolk Island, New Caledonian and Forbes' taxa). The sympatric Reischek's parakeet must have arisen from a much later, second colonisation of this island, probably by some form of red-crowned parakeet (Fleming 1979).

6.3.5 Forbes' parakeet

The taxonomy of Forbes' parakeet has long been controversial. It was originally described by Rothschild (1893) as a distinct species (*C. forbesi*) but was later relegated to a subspecies of the yellow-crowned parakeet (*C. auriceps forbesi*) by Oliver (1930) with little justification. Based on allozyme data, Triggs & Daugherty (1996) found that Forbes' parakeet is genetically quite distinct (Nei's D = 0.05) from the yellow-crowned parakeet and should again be considered as a separate species.

In the analysis presented here, Forbes' parakeet belongs to one of three distinct mitochondrial haplotype groups (see Fig. 3). Haplotype Group 1 is closely related to red-crowned parakeets, suggesting that these birds may be the result of recent hybridisation, where the female ancestor was a red-crowned parakeet. However, Forbes' parakeet HG1 is not particularly closely related to Chatham Island red-crowned parakeet haplotypes (Fig. 3), but is most similar to those

found among Little Barrier Island and Adams Island (Auckland Islands) individuals, suggesting that the apparent hybridisation event might have occurred slightly earlier than the present, (i.e. before the present Chatham Island red-crowned parakeet lineages were sorted from other haplotypes). An alternative explanation is that there could be a small proportion of parakeets in the Chatham Islands which still have mitochondrial genomes resembling some of the mainland New Zealand red-crowned parakeet lineages from which the mitochondrial haplotype of Forbes' parakeet HG1 originated. Due to the small number (five individuals) of Chatham Island red-crowned parakeets analysed, possible 'mainland'-type New Zealand red-crowned parakeet lineages may have gone undetected.

Triggs & Daugherty (1996) inferred that Forbes' parakeet was the sister taxon of red-crowned parakeets, consistent with the placement of individuals from Haplotype Group 2. Haplotype Group 2 is a sister group to the main New Zealand red-crowned parakeet clade. This haplotype group may represent a more ancient hybridisation or introgresssion event which was also directional (Chatham Island red-crowned parakeet female x Forbes' parakeet male) or might be an artifact due to general mitochondrial lineage sorting. The range of genetic divergence observed between Forbes' HG2 and individuals within the main red-crowned parakeet clade is larger than most intra-specific comparisons. This degree of differentiation may reflect an ancient introgression event shortly prior to, or coincident with, the colonisation of Mangere Island by red-crowned parakeets. The HG2 mitochondrial lineage in Forbes' parakeet has now either diverged from those in red-crowned parakeets or was already highly differentiated at the time of the original introgression event. We believe the former to be the more likely explanation because of the low level of genetic differentiation between the ancient roots of the red-crowned and Forbes' parakeets (Fig. 3).

Haplotype Group 3 in Forbes' parakeet is highly divergent with respect to all other New Zealand species in the genus *Cyanoramphus*. This haplotype group may represent the true matrilineal line of Forbes' parakeet. The existence of cryptic hybrids which are phenotypically Forbes' parakeet with mtDNA identical to or very closely resembling that of *C. n. chathamensis* (i.e. HG1 & 2) is highly probable, given what is known about the biology of the birds (see Triggs & Daugherty 1996). Mitochondrial DNA is clonally propagated and does not recombine at any stage of zygote formation; is maternally inherited and does not code for morphological traits that can be measured or observed externally. Thus, an individual may be morphologically diagnosed as Forbes' parakeet and therefore have a high percentage of Forbes' parakeet nuclear DNA, but still retain a mtDNA genome which is characteristic of *C. n. chathamensis*.

However, the issue of undiagnosed 'numts' must also be considered here. If Forbes' parakeet HG3 represents a 'numt', HG2 would then be the true Forbes' parakeet mitochondrial lineage. This latter scenario (i.e. HG3 represents a 'numt') would be more congruent with the findings and conclusions put forward by Triggs & Daugherty (1996). We cannot formally test our data to determine which scenario is correct, but the inclusion of long-PCR sequencing techniques and the diagnostic tests carried out on the DNA sequences (see methods) make it unlikely that our data have originated from nuclear pseudogenes. Based on our original hypothesis, i.e. that HG3 is the true Forbes'

parakeet lineage, the conflict with data presented by Triggs & Daugherty (1996) is more apparent than real if one admits the possibility of cytonuclear disequilibrium. The study by Triggs & Daugherty (1996) is based on nuclear markers alone which are less discriminatory than mitochondrial control region data and may reflect only partially differentiated gene pools between Forbes' and Chatham Island red-crowned parakeets—due in part to historical hybridisation. At the time of writing, we are confident that the HG3 haplotype represents the true Forbes' parakeet gene pool; however, highly discriminating nuclear microsatellite markers will be required to elucidate the historical mechanisms that gave rise to this complex pattern of genetic differentiation.

6.4 PHYLOGENETIC ANALYSES

The analyses performed here have produced extremely robust phylogenies which were well supported by bootstrap resampling. The three different methods: parsimony, distance and likelihood produced phylogenies that were congruent with one another. The topology was also insensitive to changes in nucleotide substitution models, although it was found that correction for among-site rate variation was essential in phylogenetic reconstructions. This is due in part to the heterogeneous nature of the three domains of the mitochondrial control region which evolve at different rates, have different base compositions and are under different structure to function constraints (see Quinn 1997). The assumption that certain sites are invariable (I) did not seem to affect the likelihood scores of a given inferred topology greatly, but incorporation of gamma distribution corrections for the variable sites (G) did. We believe that the phylogenies of the full 73-taxon dataset (parsimony and neighbour-joining) and reduced dataset (maximum likelihood and minimum evolution) are robust and reflect the dataset accurately.

The time-constrained tree (Fig. 5) was constructed from sequences of control region domains 2 + 3 and not from 2 alone, although the latter does behave in a time-constrained manner. That is because, when data from domain 2 alone is used to construct a phylogenetic tree, there is a severe loss of tree structure and fine-scale phylogenetic information due to its high sequence conservation. The time-constrained tree presented for domains 2 + 3 combined does have sufficient phylogenetic information.

6.5 MOLECULAR HISTORY OF *Cyanoramphus*

Taylor (1975) proposed that the New Zealand Kakariki parakeets we see today evolved from a common ancestral form during the Pleistocene (between 20 000 and 2 000 000 years b.p.) when fluctuating ice and sea barriers favoured speciation in isolated refugia. With our extensive reclassification of the *Cyanoramphus* taxa, it is timely to re-interpret the evolutionary history of this genus based on all the available molecular data. Our estimates for the timing of evolutionary events presented in this section, as well as in Section 5.3, can only serve as a rough guide to the true timing of these events. They are perhaps best and most reliably considered relative to one another than as absolute dates (Hillis et al. 1996).

The Kakariki has always been considered to be a New Zealand endemic which is a modern version of a possible proto-*Cyanoramphus* taxon originating from the supercontinent of Gondwana. It followed that the nearest living relative of *Cyanoramphus* was probably one of the Australian parrot taxa; the most probable being the Rosella group (*Platycercus* spp.) which is morphologically closest to the New Zealand *Cyanoramphus*. Falla (1953) also proposed that Australia has been the source of the majority of New Zealand's birds. Using molecular dating methods, we propose a theory extending the concept of an Australian origin via a New Caledonian staging post. This idea was motivated by the basal placement of the New Caledonian red-crowned parakeet in the *Cyanoramphus* complex (Fig. 3) and new developments in our understanding of the geological history of the area.

Palaeogeographic reconstructions show that Oligocene uplift of the Norfolk Ridge and Miocene uplift of the Reinga Ridge could have provided a means for terrestrial biota with New Caledonian affinities to spread into New Zealand in the early and middle Miocene (Herzer et al. 1997). According to Herzer et al. (1997), a continuous landmass was formed during the mid-Cretaceous rifting of the Norfolk Ridge and was later cut into a chain of long islands separated by short stretches of water. The Southern end of the Norfolk Ridge was later uplifted, extending to 32°S, followed by a series of island chains formed by rising of the Reinga Ridge towards Northland (Herzer et al. 1997; Mortimer et al. 1998). It is therefore possible for New Caledonian taxa to have colonised New Zealand via these islands as they formed.

The time of divergence estimated for the *Eunymphicus / Platycercus* split allows consideration of the possibility that they may have shared a common ancestor prior to the separation of Gondwanaland. The 20 Myr (see Appendix 2) dating may be an underestimate because transition substitutions were not taken into account. Given the error rate involved in such estimates, the separation may well have occurred any time from the mid Oligocene, perhaps even extending back towards the beginning of the Paleocene epoch (30–65 Myr b.p.). The closest relative of *Eunymphicus* may not even be *Platycercus*, as the level of divergence from *Eunymphicus* to *Platycercus* (P-dist = 0.13 for cytochrome *b*, 924 sites compared) is approximately equal to divergences between *Eunymphicus* and several of the Australian parrot (e.g. *Eunymphicus* versus *Geopsittacus*, P-dist = 0.15 for cytochrome *b*, 924 sites compared) taxa presented by Leeton et al. (1994).

Our data strongly support Smith (1975) that *Eunymphicus* is the closest living relative of *Cyanoramphus*. The divergence from *Cyanoramphus* to *Eunymphicus* is much lower than from *Platycercus* to *Cyanoramphus* (giving dates 2.9 Myr b.p. versus 20.1 Myr b.p.). The colonisation of New Zealand by proto-*Cyanoramphus* could have been made possible by the North to South formation of island chains from New Caledonia towards Northland. The presence of *Eunymphicus* only in New Caledonia suggests that *Cyanoramphus* must have diverged from the *Eunymphicus-Cyanoramphus* common ancestor before colonising Norfolk Island and, finally, New Zealand and the subantarctic islands. From our data, the time taken for the New Caledonian red-crowned parakeet to colonise and speciate into the current radiation is 450 000–625 000 years, a value which does not correspond to the proposed Miocene (23–5 Myr b.p.) uplift of the Reinga Ridge (Herzer et al. 1997).

Although geological and molecular time estimates are both only approximate and may well overlap each other, we acknowledge that the time estimates (geological: 23–5 Myr b.p.; molecular: 450 000–600 000 yrs b.p.) are different by many orders of magnitude. Although the 'land bridge' theory of colonisation is intriguing, it may not be a viable explanation. The alternative explanation would be colonisation of New Zealand by dispersal of *Cyanoramphus* parakeets from New Caledonia via Norfolk Island.

The dispersalist hypothesis is highly likely given the structure and magnitude of the genetic variation observed within the genus *Cyanoramphus*. The proposed Forbes' parakeet HG3 / Caledonian red-crowned parakeet split was inferred to have occurred 441 000 years b.p. while all other New Zealand taxa had common ancestors ranging from 279 000–14 000 years b.p., which suggests the possibility of early colonisation of the Chatham Islands by proto-*Cyanoramphus*, giving rise to the modern Forbes' parakeet (see Fig. 5). With an earlier split of the Forbes' parakeet lineage relative to the Norfolk Island red-crowned parakeet, one would have to assume that the first population that colonised this location perished. The population of Norfolk Island *Cyanoramphus* currently observed would then be the result of 'back-colonisation' from New Zealand after *Cyanoramphus* became established in New Zealand. It seems likely that the red crown colouration seen across many lineages of the *Cyanoramphus* complex (particularly the older ones) is an ancestral character, which has changed numerous times during the evolution of this genus. The labile nature of crown-colouration character in this genus has produced several new species of birds with either a yellow crown (*C. auriceps, C. forbesi*) or yellow crown with orange frontal band (*C. malherbi*), or loss of all crown colouration to produce a uniformly green-headed bird (*C. unicolor*). The ancestral red colouration has been retained in *C. novaezelandiae, C. erythrotis, C. saisetti* and *C. cooki*.

The Chatham Islands were colonised later by the red-crowned parakeet. The explosive adaptive radiation of the *Cyanoramphus* genus was probably assisted by fluctuating glacial and interglacial episodes during the Pleistocene, which may have isolated a single population (or populations) into multiple allopatric 'refugia'. The periodic linking of the main islands of New Zealand to each other and to near-shore islands, plus the persistent separation of other offshore islands, has led to some degree of endemism, especially in the North where climatic deterioration at each glacial stage was not sufficiently severe to eliminate the fauna and flora (Fleming 1979).

Fleming (1979) proposed that whatever New Zealand flora and fauna had developed by the late Pliocene must have been decimated in the Pleistocene when permanent snow reached below 350 m. Woody vegetation was diminished and North Canterbury and Southland had a barren, treeless landscape. Moar (1971) concluded that modern *Nothofagus* forest was not established in the South Island before 10 000 years ago, while McGlone (1973), McGlone & Topping (1973) and McGlone & Howorth (1976) described the presence of grassland and scrub vegetation with a mosaic of *Nothofagus* in the central North Island and the current coast of Opotiki at about the same time. The predominance of grass and scrub vegetation habitat in the glacial and post-glacial periods may be reflected by the present lifestyle of one of the more ancient *Cyanoramphus* lineages (*C. unicolor*) which lives in tussock and sedge

grass habitats in the Antipodes Island. This characteristic may be an ancestral (plesiomorphic) character which was lost when native New Zealand forest became established after the retreat of the ice at around 10 000–14 000 years b.p. and mainland New Zealand species occupied the new habitat. The dates for the ice retreat are discussed in Stevens (1980).

The types of vegetation described by McGlone (1973), McGlone & Topping (1973) and McGlone & Howorth (1976) for mainland New Zealand before the ice retreated resemble that of the current Antipodes Island (green) parakeet (*C. unicolor*) habitat, while all other *Cyanoramphus* species which inhabit the mainland of New Zealand and those offshore islands which are not sub-antarctic, do so in tracts of forest. The sympatric Reischek's parakeet (*C. e. hochstetteri*) lives in a habitat similar to that of the Antipodes Island (green) parakeet, probably as a secondary adaptive feature, rather than a retained ancestral character. The Macquarie Island red-crowned parakeet (*C. erythrotis*) also lived in habitats similar to those occupied by Reischek's parakeet (*C. e. hochstetteri*). We take this as further support for our genetic hypothesis which suggests that Macquarie Island may have been colonised by proto-Reischek's parakeet, giving rise to the Macquarie Island taxon. The reversed scenario would, however, be less likely.

The Chatham Island red-crowned (*C. n. chathamensis*) and Reischek's parakeets (*C. e. hochstetteri*) appear to stem from the mainland New Zealand stock of the red-crowned parakeet (20 000–50 000 years b.p.) and are the results of independent colonisation events, as postulated by Fleming (1952) and Taylor (1975). The orange-fronted parakeet (*C. malherbi*) may have resulted from a vicariant event as an isolated population of the New Zealand red-crowned parakeet population during the glacial-interglacial fluctuations, allowing allopatric speciation to occur.

From historical records, it is certain that yellow, red and orange-fronted parakeets occurred widely over the mainland of New Zealand. This may be the result of widespread re-establishment of large tracts of forest during the last 10 000 years. The current limited distribution of *Cyanoramphus* is mostly due to the result of the arrival of humans and their activities which fragmented the habitats of these birds and rendered some populations extinct. Two further extinct taxa—the black-fronted parakeet (*C. zelandicus*) and Society parakeet (*C. ulietanus*)—have not yet been analysed. The DNA extracted from these specimens was too degraded to be used as good templates for PCR amplification but small amplification products may be obtained for DNA sequencing. Data obtained from these two taxa may enable us to confirm whether these taxa represent the most ancient Kakariki lineages, ancient radiations from New Caledonia or recent radiations from New Zealand.

7. Conclusions

Our genetic survey provides sufficient evidence to recommend increasing the number of *Cyanoramphus* species from five (*C. auriceps, C. novaezelandiae, C. ulietanus, C. unicolor* and *C. zelandicus*) (Higgins 1999) to ten. They are:

- Orange-fronted parakeet (*C. malherbi*)
- Forbes' parakeet (*C. forbesi*)
- Macquarie Island Red-crowned parakeet (*C. erythrotis*)
- Norfolk Island red-crowned parakeet (*C. cooki*)
- New Caledonian red-crowned parakeet (*C. saisetti*)

} New classification

- Yellow-crowned parakeet (*C. auriceps*)
- Antipodes Island (green) parakeet (*C. unicolor*)
- New Zealand red-crowned parakeet (*C. novaezelandiae*)
- Society parakeet (*C. ulietanus*)
- Black-fronted parakeet (*C. zelandicus*)

} Classification unchanged

The subspecies are listed below as:

- Reischek's parakeet (*C. e. hochstetteri*)

} New classification

- Chatham Island re-crowned parakeet (*C. n. chathamensis*)
- Kermadec Island red-crowned parakeet (*C. n. cyanurus*)
- Lord Howe Island red-crowned parakeet (*C. n. subflavescens*)

} Classification unchanged

The Chatham Island red-crowned parakeet (*C. n. chathamensis*) remains a subspecies of the mainland New Zealand red-crowned parakeet but Reischek's parakeet (*C. e. hochstetteri*) has been reclassified as a subspecies of the Macquarie Island red-crowned parakeet (*C. erythrotis*). We cannot presently comment with confidence on the status of the Kermadec Island red-crowned parakeet (*C. n. cyanurus*) or the extinct Lord Howe Island red-crowned parakeet (*C. n. subflavescens*), and recommend retaining the present status of the black-fronted parakeet (*C. zelandicus*) and Society parakeet (*C. ulietanus*). In any event, they are likely to remain as full species given their geographical separation from the main *Cyanoramphus* population and their morphological distinctiveness. Their present names are also relatively neutral with respect to phylogenetic implication. The following represent the key findings from our study:

- The mtDNA cytochrome *b* gene analysis system developed here provides robust phylogenetic information only at deeper divergences (genus and above) for parrot species if the sample sizes are high.
- Patterns of genetic variation at the mtDNA control region of the orange-fronted parakeet, Macquarie Island red-crowned parakeet, Forbes' parakeet, New Caledonian red-crowned parakeet and Norfolk Island red-crowned parakeet fulfil the criteria of the Phylogenetic Species Concept and support full independent species status for these taxa as *C. malherbi, C. erythrotis, C. forbesi, C. saisetti* and *C. cooki* respectively.
- Forbes' parakeet possesses three main mtDNA haplogroups. One (HG1) is proposed as a result of recent hybridisation with a red-crowned parakeet (see Section 6.3 on Forbes' parakeet). A second (HG2) is an older lineage originating from a more ancient introgression event with the same species and a third,

highly divergent haplogroup (HG3) which probably represents the original Forbes' parakeet matriline from an early radiation of *Cyanoramphus*.

- The existence of cryptic hybrids which are phenotypically Forbes' parakeets (*C. forbesi*) but with mtDNA and nuclear markers identical to or very closely resembling that of *C. n. chathamensis* is highly probable.
- Hybridisation between Chatham Island red-crowned parakeet and Forbes' parakeet may be unidirectional. If hybridisation is unidirectional (i.e. female Chatham Island red-crowned parakeet x male Forbes' parakeets), it would only be possible to find phenotypically Forbes' parakeet with Chatham Island red-crowned parakeet matrilines. Our limited mtDNA sequence data (11 Forbes' and 5 Chatham Island red-crowned parakeets) currently points toward this hypothesis, but due to the small sample size we cannot confirm this assumption.
- Our data lack the resolution required to support the conclusion of Triggs & Daugherty (1996) that there is a separation of yellow-crowned parakeet (*C. auriceps*) into northern and southern groups. More work is required to confirm this proposal.
- Reischek's parakeet (*C. e. hochstetteri*) is a subspecies of Macquarie Island red-crowned parakeet (*C. erythrotis*), not of the New Zealand red-crowned parakeet (*C. novaezelandiae*).
- The sister species of the orange-fronted parakeet (*C. malherbi)* is not the yellow-crowned parakeet (*C. auriceps*) but rather Reischek's and the Macquarie Island red-crowned parakeet (*C. e. hochstetteri* and *C. erythrotis* respectively).
- The Kakariki may have arisen from the Australian *Platycercines* via proto-*Eunymphicus* in New Caledonia.
- Molecular data suggests that it is likely that New Zealand *Cyanoramphus* parakeets dispersed from New Caledonia to New Zealand via Norfolk Island very recently within the last 450 000–625 000 years.
- The most ancient lineage of *Cyanoramphus* is the New Caledonian endemic *C. saisetti* which is the likely source of the radiation of species in New Zealand and its offshore islands. This was probably the result of vicariant events due to the fluctuations in glacial ice followed by allopatric speciation in the last 450 000–625 000 years.
- Most of mainland New Zealand and its offshore islands may have been re-colonised by emergent *Cyanoramphus* taxa after speciation events during the last 14 000 years (in the late-Pleistocene / early-Holocene) after glacial ice retreated and forests began to re-establish.
- The Antipodes Island was colonised twice by *Cyanoramphus* species, as originally postulated by Fleming (1952). The first colonisation was by proto-Antipodes Island (green) parakeet (*C. unicolor*) at about 280 000 years b.p. and the second by ancestors of modern stock of Reischek's parakeet (*C. e. hochstetteri*) about 30 000 years b.p.
- Chatham Islands were also colonised twice by *Cyanoramphus* species. The modern Forbes' parakeet (*C. forbesi*) was derived from an ancient colonisation by *Cyanoramphus* at approximately 440 000 years b.p. and the Chatham Island red-crowned parakeet (*C. n. chathamensis*) is derived from a more recent colonising event about 60 000 years b.p.

- The global phylogeny proposed for the *Cyanoramphus* complex of species in this report is robust and insensitive to variations in tree-building optimality criteria or substitution models.
- The incorporation of among-site rate variation corrections are important for phylogenetic reconstruction of the *Cyanoramphus* phylogeny based on the mitochondrial control region sequences.

8. Recommendations

The newly proposed *Cyanoramphus* species (*C. malherbi, C. erythrotis, C. forbesi, C. saisetti* and *C. cooki*) are all currently endangered and are localised on extremely small segments of mainland New Zealand or on offshore islands in the Southern Pacific ocean (except for *C. erythrotis* which is extinct). It is important to note that the following species-level recommendations should also extend to Reischek's parakeet (*C. e. erythrotis*) as it is the only remaining lineage which is closely related to the extinct Macquarie Island red-crowned parakeet (*C. erythrotis*). They are thus highly sensitive to any fluctuations in their environment, be it the introduction of predators, destruction of habitat by fires or other factors, climatic changes affecting availability of food or a combination of these. Another factor affecting the long-term viability of some species and populations is genetic swamping of a rare taxon, as exemplified by Forbes' parakeet on Mangere Island. Better knowledge of *Cyanoramphus* diversity and evolution can allow better-informed steps to be taken to assist in the conservation management of these birds. The combination of genetic and ecological data provides us with a powerful tool with which 'enlightened' conservation decisions may be made.

One of the major difficulties faced in interpretation of mitochondrial DNA data for conservation management is its strict maternal mode of inheritance. Surveying genetic markers on this molecule does not provide a direct measure of the genetic variation within the nuclear genome of the organism, but rather provides information about persistence of maternal lineages. Our data support the need for further characterisation of the nuclear genes of these birds. We also recommend studying the ecology of Forbes' parakeet in detail to provide a better understanding of factors responsible for the widespread hybridisation which has occurred in the past.

The orange-fronted, Forbes', New Caledonian red-crowned and Norfolk Island red-crowned parakeets are full species. While Reischek's parakeet is taxonomically classified as a subspecies, it represents the lineage of a full species—the extinct Macquarie Island red-crowned parakeet. Ecological and behavioural studies are also needed urgently for all of these species and population numbers should be monitored. Recovery plans should also be made or re-evaluated in light of the new genetic information in order to enable the most effective protection of these species at stable population sizes. When the microsatellite techniques presently being developed for Forbes' parakeet become available, a wider genetic survey of other populations of *Cyanoramphus* species should be conducted. Given the initial investment in

developing microsatellite markers for Forbes' parakeet, other *Cyanoramphus* species can then be surveyed in a relatively cost-effective manner with considerable benefit to conservation management. The population of *Cyanoramphus* parakeets on the Auckland Islands should be given high priority in terms of commissioning an allozyme / mtDNA / microsatellite analysis survey in order to genetically characterise the apparent hybrid swarm of red x yellow-crowned parakeets that currently exists on that island. Additionally, the Auckland Islands yellow-crowned parakeet population may well represent a novel and genetically distinct species or subspecies of the yellow-crowned parakeet. Its situation would be reminiscent of the other 'yellow-crowned' offshore island dweller, Forbes' parakeet, on Mangere Island. The same level of scrutiny should also be applied to the red-crowned parakeet population on Auckland Island which may be a distinct subspecies (cf. *C. n. chathamensis*) or species (cf. *C. erythrotis*).

Further ancient DNA trials on specimens for Society (*C. ulietanus*) and black-fronted (*C. zelandicus*) parakeets may confirm whether they are part of the New Zealand radiation of *Cyanoramphus* parakeets or represent the two most ancient lineages in the genus after the New Caledonian red-crowned parakeet. The two former species may well be more closely related to the New Caledonian *Eunymphicus* genus than the New Caledonian red-crowned parakeet given their morphological similarities, particularly with respect to plumage colouration. Equally, analysis of the North Island orange-fronted parakeet may confirm whether the mitochondrial haplotype of the yellow-crowned parakeet from Little Barrier Island (FT1018) represents the northern haplotype of the orange-fronted parakeet. Overall, the study presented here shows the potential for mtDNA to resolve phylogenies, delineate species, probe hybridisation events and inform conservation managers regarding the status of gene pools present in these species.

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Appendix 1

DETAILS OF BIRDS SAMPLED AND TYPE OF SAMPLE TAKEN

TABLE A1.1. DETAILS OF BIRDS SAMPLED AND SAMPLES TAKEN.

NUMBER	COMMON NAME	SCIENTIFIC NAME	SPECIMEN CODE	LOCALITY
1	Antipodes Island parakeet	*C. unicolor*	CD1130	Antipodes Island, N.Z. (B)
2			CD965	Antipodes Island, N.Z. (B)
3			CD966	Antipodes Island, N.Z. (B)
4			CD967	Antipodes Island, N.Z. (B)
5	New Zealand red-crowned parakeet	*C. novaezelandiae novaezelandiae*	CD1212	Captive: Nga Manu Wildlife Sanctuary, North Island, N.Z.,unknown origin. (B)
6			AD1	Adams Island, Auckland Islands Group, N.Z. (F)
7			EN1	Enderby Island, Auckland Islands Group, N Z. (F)
8			CF1	Codfish Island, Southern South Island, N.Z. (F)
9			CF2	Codfish Island, Southern South Island, N.Z. (F)
10			CD2035	Poor Knights Island, Northern North Island, N.Z. (B)
11			CD2038	Poor Knights Island, Northern North Island, N.Z. (B)
12			FT1016	Little Barrier Island, Northern North Island, N.Z. (B)
13	Chatham Island red-crowned parakeet	*C. n. chathamensis*	CD1843	Rangatira Island, Chatham Islands, East of N.Z. mainland. (B)
14			CD1838	Rangatira Island, Chatham Islands, East of N.Z. mainland. (B)
15			RA4	Rangatira Island, Chatham Islands, East of N.Z. mainland. (F)
16			PK23	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
17			PK24	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
18	Reischek's parakeet	*C. e. hochstetteri*	CD968	Antipodes Island, N.Z. (B)
19			CD969	Antipodes Island, N.Z. (B)
20			RE1	Antipodes Island, N.Z. (F)
21			RE2	Antipodes Island, N.Z. (F)
22			RE3	Antipodes Island, N.Z. (F)
23			RE4	Antipodes Island, N.Z. (F)
24	Norfolk Island red-crowned parakeet	*C. cooki*	C1	Norfolk Island, Australia. (F)
25			C4	Norfolk Island, Australia. (F)
26			C5	Norfolk Island, Australia. (F)
27	New Caledonian red-crowned parakeet	*C. saisetti*	RCP00071	New Caledonia, France. (B)
28			RCP00129	New Caledonia, France. (B)
29			RCP00131	New Caledonia, France. (B)
30			RCP00132	New Caledonia, France. (B)
31	Macquarie Island red-crowned parakeet	*C. erythrotis*	AV2099	Macquarie Island, Australia. (F)
32	Forbes' Parakeet	*C. forbesi*	PMC26	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
33			CD1810	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
34			CD1811	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
35			CD1814	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
36			CD1815	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
37			CD1816	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
38			CD1817	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)

TABLE A1.1 *Continued.*

NUMBER	COMMON NAME	SCIENTIFIC NAME	SPECIMEN CODE	LOCALITY
39	Forbes' Parakeet	*C. forbesi*	CD1818	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
40			CD1819	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
41			CD1820	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
42			CD1821	Mangere Island, Chatham Islands, East of N.Z. mainland. (B)
43	Yellow-crowned Parakeet	*C. auriceps*	FT3303	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
44			FT3304	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
45			FT3305	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
46			FT3306	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
47			FT3307	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
48			FT3310	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
49			FT3311	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
50			FT3323	Eglinton Valley, Fiordland National Park, South Island, N.Z. (B)
51			FT3324	Eglinton Valley, Fiordland National Park, South Island, N.Z. (B)
52			FT3325	Eglinton Valley, Fiordland National Park, South Island, N.Z. (B)
53			WG168	Eglinton Valley, Fiordland National Park, South Island, N.Z. (B)
54			FT1018	Little Barrier Island, Northern North Island, N.Z. (B)
55			FT1029	Little Barrier Island, Northern North Island, N.Z. (B)
56			FT1874	Chetwode Islands, Northern South Island, N.Z. (B)
57			FT1878	Chetwode Islands, Northern South Island, N.Z. (B)
58			PSF1	Pureora State Forest Park, Central North Island, N.Z. (F)
59			PSF2	Pureora State Forest Park, Central North Island, N. Z. (F)
60			FT3308	Hawdon Valley, Arthur's Pass National Park, South Island, N.Z. (L) (B)
61			AV49	Pipiriki, North Island, N.Z. (F)
62			AV62	Pipiriki, North Island, N.Z. (F)
63			AV56	Ohakune, North Island, N.Z. (F)
64			AV47	Ohakune, North Island, N.Z. (F)
65	Orange-fronted parakeet	*C. malherbi*	FT3309	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
66			FT3312	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
67			FT3314	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
68			FT3315	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
69			FT3316	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
70			FT3317	South Branch Hurunui Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
71			WG611	Hope Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
72			WG612	Hope Valley, Lake Sumner Forest Park, South Island, N.Z. (B)
73			IS1	Captive: Isaac's Aviary, Christchurch. Originated from Hope Valley, Lake Sumner Forest Park, South Island, N.Z. (F)
74			AW1	Captive: Alan Wright's aviary, Nelson, N.Z. Originated from breeding experiments by Taylor et al. (1986). (F)

TABLE A1.1 *Continued.*

NUMBER	COMMON NAME	SCIENTIFIC NAME	SPECIMEN CODE	LOCALITY
75	Orange-fronted parakeet	*C. malherbi*	AW2	Captive: Alan Wright's aviary, Nelson, N.Z. Originated from breeding experiments by Taylor et al. (1986). (F)
76			JK1	Captive: Jonathan Kearvell's aviary, Rangiora, N.Z. Second generation progeny of a Hope Valley female. (F)
77			AV63	Owen Junction, Nelson, N.Z. (F)
78			2447	Owen Junction, Nelson, N.Z. (F)
79			O.30426	Drury, North Island, N.Z. (F)
80			7869	Otago or Nelson, N.Z.—uncertain. (F)
81			30.383	Taranga Island, North Island, N.Z. (F)
82			50.379	Chalky Sound, South Island, N.Z. (F)
83			50.380	Chalky Sound, South Island, N.Z. (F)
84			50.381	Mount Foster, Dusky Sound, South Island, N.Z. (F)
85			50.382	Taranga Island, North Island, N.Z. (F)
86			50.387	Chalky Sound, South Island, N.Z. (F)
87	Black-fronted parakeet	*C. zelandicus*	1859.11.22.20	Society Islands. (F)
88	Society parakeet	*C. ulietanus*	VEL 22.31.a	Society Islands. (F)
89	Red x Yellow-crowned Parakeet Hybrid	N/A	FT1010	Little Barrier Island, Northern North Island, N.Z. (B)
90			FT1013	Little Barrier Island, Northern North Island, N.Z. (B)

All samples analysed and included in this report are listed above. The scientific names correspond to each taxa as recognised in this report after reconsideration of their taxonomic status using the molecular genetic data presented here. The tissue type used for analysis are abbreviated as follows: (L) Liver, (B) Blood, (F) Feather

Appendix 2

TIME OF DIVERGENCE ESTIMATES

TABLE A2.1. TIME OF DIVERGENCE ESTIMATES FOR PARAKEET GENERA AND SPECIES.

TAXA COMPARED	NUMBER TS	NUMBER TV	PROPORTION DIFFERENT	TOTAL NUMBER OF SITES COMPARED	TIME DIVERGENCE ESTIMATE (MYR)
Melopsittacus v. *Geopsittacus*	68	52	0.13	924	30.37
Melopsittacus v. *Neophema*	74	54	0.14	924	31.76
Melopsittacus v. *Platycercus*	61	43	0.11	924	24.91
Melopsittacus v. *Cyanoramphus* (AGP)	67	51	0.13	924	29.75
Melopsittacus v. *Eunymphicus*	69	50	0.13	924	29.24
Melopsittacus v. *Cyanoramphus* (YCP)	68	51	0.13	924	29.79
Melopsittacus v. *Cyanoramphus* (NcRCP)	72	50	0.13	924	29.34
Geopsittacus v. *Neophema*	87	58	0.16	924	34.65
Geopsittacus v. *Platycercus*	75	57	0.14	924	33.57
Geopsittacus v. *Cyanoramphus* (AGP)	72	63	0.15	924	36.97
Geopsittacus v. *Eunymphicus*	71	64	0.15	924	37.51
Geopsittacus v. *Cyanoramphus* (YCP)	73	63	0.15	924	37.02
Geopsittacus v. *Cyanoramphus* (NcRCP)	71	62	0.14	924	36.34
Neophema v. *Platycercus*	81	47	0.14	924	27.88
Neophema v. *Cyanoramphus* (AGP)	92	51	0.15	924	30.65
Neophema v. *Eunymphicus*	88	50	0.15	924	29.90
Neophema v. *Cyanoramphus* (YCP)	93	51	0.16	924	29.21
Neophema v. *Cyanoramphus* (NcRCP)	89	50	0.15	924	29.94
Platycercus v. *Cyanoramphus* (AGP)	80	34	0.12	924	20.14
Platycercus v. *Eunymphicus*	78	35	0.12	924	20.69
Platycercus v. *Cyanoramphus* (YCP)	79	34	0.12	924	20.12
Platycercus v. *Cyanoramphus* (NcRCP)	77	33	0.12	924	19.48
Cyanoramphus (AGP) v. *Eunymphicus*	58	5	0.07	924	2.89
Cyanoramphus (AGP) v. *Cyanoramphus* (YCP)	6	0	0.01	1140	-
Cyanoramphus (AGP) v. *Cyanoramphus* (NcRCP)	28	1	0.03	1140	0.45
Eunymphicus v. *Cyanoramphus* (YCP)	58	5	0.07	924	2.89
Eunymphicus v. *Cyanoramphus* (NcRCP)	58	4	0.07	924	2.31
Cyanoramphus (YCP) v. *Cyanoramphus* (NcRCP)	28	1	0.03	1140	0.45

The time of divergence estimates are evaluated based on the total number of transversions only. The rate of evolution adopted for calculation is 0.20% per million years (Irwin et al. 1991). Time of divergence = [tv/(total number of sites compared - ts)] × (0.20 %/Myrs).