

Molecular systematics of New Zealand *Cyanoramphus* parakeets: conservation of Orange-fronted and Forbes' Parakeets

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Summary

The controversy that presently surrounds the taxonomy of the Orange-fronted *Cyanoramphus malherbi* and Forbes' *C. forbesi* Parakeets has important implications for the conservation of both birds. Both taxa are critically endangered, but consensus regarding their specific status has not yet been achieved. We present mitochondrial DNA sequences for the cytochrome *b* gene and the control region from 17 *Cyanoramphus* parakeets representing nine populations and six taxa together with field observations of courtship and breeding behaviour in a sympatric population of Orange-fronted and Yellow-crowned Parakeets *C. auriceps*. Field data support species status of the Orange-fronted Parakeet under the Biological Species Concept. Phylogenetic analyses of our DNA sequence data support earlier hypotheses based on allozyme data that both Orange-fronted and Forbes' Parakeets represent distinct species under four species concepts and indicate that high conservation priority is warranted for both taxa.

Introduction

Parakeets of the genus *Cyanoramphus* (Psittacidae: Psittacinae) occur in the South Pacific from the tropics to the subantarctic (Taylor 1985) with their distribution centred in New Zealand (Figure 1, Table 1). Most taxa have limited ranges and are thus vulnerable to extinction (Taylor 1985). Most previous taxonomies have recognized three extant species and six subspecies of New Zealand *Cyanoramphus* parakeets (Turbott 1990), but a recent genetic study by Triggs and Daugherty (1996) claimed that five extant species should be recognized, elevating two of the rarest and most endangered forms, Forbes' Parakeet and Orange-fronted Parakeet to full species status: *C. forbesi* and *C. malherbi* respectively. However, this view has subsequently been challenged by Taylor (1998). In this study, we follow the *Cyanoramphus* parakeet nomenclature as recommended by Triggs and Daugherty (1996).

Conservation and taxonomy

Poor taxonomy can compromise conservation management. Avise and Nelson (1988) showed several instances where faulty taxonomy has resulted in well-intentioned, but misdirected, efforts in endangered species management. An example of inconsistent conservation strategies for endangered New Zealand

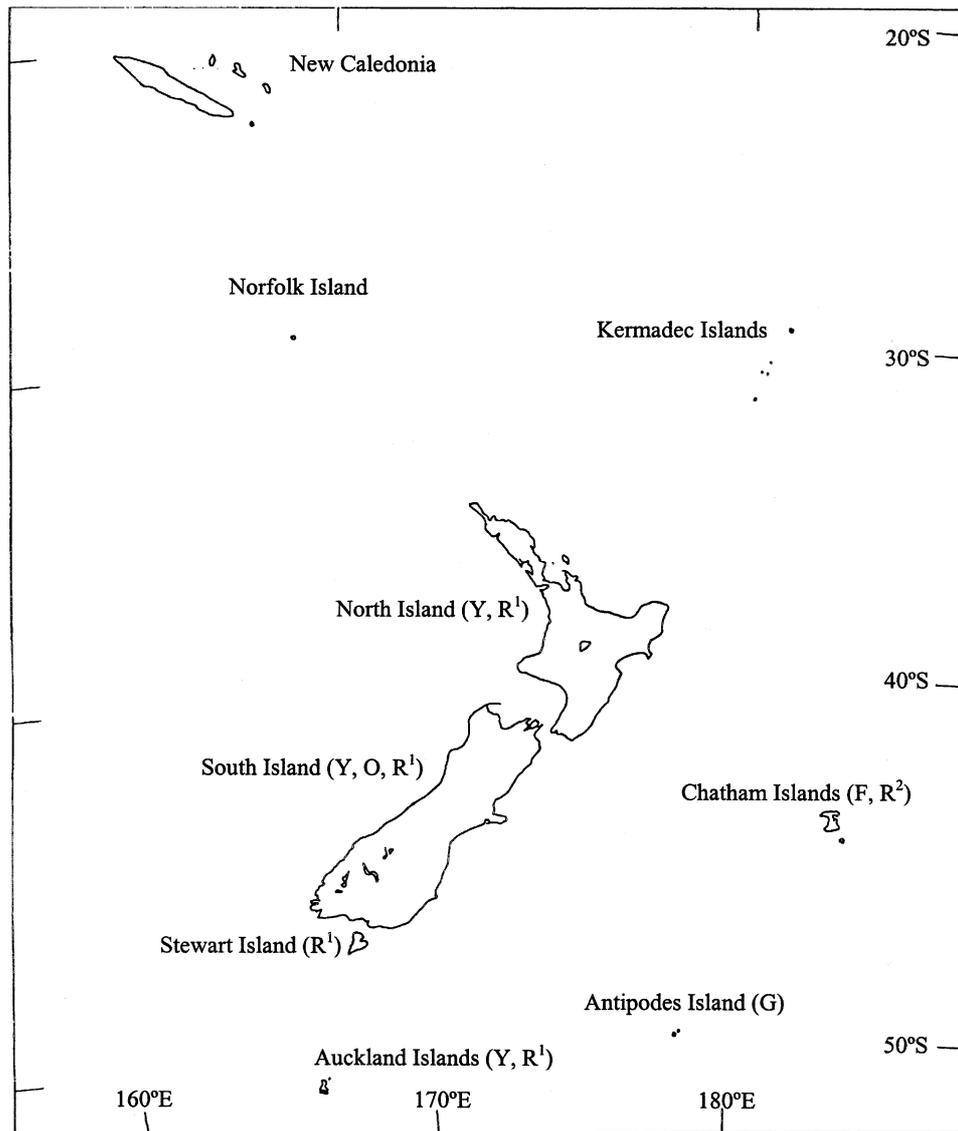


Figure 1. Distribution of *Cyanoramphus* parakeets examined in this study. G, Antipodes Island (Green) Parakeet *C. unicolor*; O, Orange-fronted Parakeet *C. malherbi*; Y, Yellow-crowned Parakeet *C. auriceps*; F, Forbes' Parakeet *C. forbesi*; R¹, New Zealand Red-crowned Parakeet *C. novaezelandiae novaezelandiae*; R², Chatham Island Red-crowned Parakeet *C. n. chathamensis*.

parakeets is exemplified by the intense management of Forbes' Parakeet, which was officially classified as a subspecies, while the Orange-fronted Parakeet, which is a species (Triggs and Daugherty 1996), has not received anywhere near the same level of attention. There were other extenuating circumstances affecting the unequal level of attention both species received, the most prominent being

Table 1. Species and subspecies of *Cyanoramphus* parakeets (Higgins 1999, Forshaw 1989, Taylor 1985)

Common name	Scientific name
Antipodes Island (Green) Parakeet	<i>C. unicolor</i>
Orange-fronted Parakeet	<i>C. malherbi</i>
Yellow-crowned Parakeet	<i>C. auriceps</i>
Forbes' Parakeet	<i>C. forbesi</i>
Red-crowned Parakeet	<i>C. novaezelandiae novaezelandiae</i>
Chatham Island Red-crowned Parakeet	<i>C. n. chathamensis</i>
Reischek's Parakeet	<i>C. n. hochstetteri</i>
Kermadec Parakeet	<i>C. n. cyanurus</i>
New Caledonia Red-crowned Parakeet	<i>C. n. saisetti</i>
Norfolk Island Red-crowned Parakeet	<i>C. n. cooki</i>
Lord Howe Island Red-crowned Parakeet (extinct)	<i>C. n. subflavescens</i>
Macquarie Island Red-crowned Parakeet (extinct)	<i>C. n. erythrotis</i>
Black-fronted Parakeet (extinct)	<i>C. zelandicus</i>
Society Parakeet (extinct)	<i>C. ulietanus</i>

the difficulty in locating and identifying Orange-fronted Parakeets. When good taxonomic data are available, they should always form the basis upon which conservation decisions are based, and molecular genetic information should be an integral part of such data, wherever possible. As shown above, the comparatively inconsistent management of Forbes' and Orange-fronted Parakeets is a direct result of the lack of detailed genetic information on which to base decisions.

History of Orange-fronted Parakeet

The Orange-fronted Parakeet has had a complex taxonomic history since the mid-1800s (Harrison 1970). It was formally described by Souance (1857) as *Cyanoramphus malherbi* and later as *Platycercus malherbi* by Gray (1862) and as the young of *Platycercus auriceps* by Finsch (1869). Buller (1869) described it as the Alpine Parrot *Platycercus alpinus*. It was known by this name until Salvadori (1891) synonymized Buller's name for the species with that first given by Souance. From then on, it was considered a separate species as *C. malherbi* (Oliver 1930, 1955, Falla *et al.* 1966). Harrison (1970) reviewed its full history and again supported Souance's original classification of the Orange-fronted Parakeet as a species on its own.

Since then, the specific status of the Orange-fronted Parakeet has been the subject of continual debate. Based on morphology and limited field observations, Holyoak (1974) considered the Orange-fronted Parakeet a colour morph of sympatric Yellow-crowned Parakeets *C. auriceps*. This was supported by preliminary morphological data (Nixon 1981) and captive breeding experiments (Taylor *et al.* 1986). However, based on their allozyme electrophoresis data, Triggs and Daugherty (1996) questioned the "colour-morph" hypothesis by showing the Orange-fronted Parakeet to be genetically well differentiated from both sympatric and geographically distant South Island Yellow-crowned Parakeets. These taxa showed about the same level of differentiation as was found among well-accepted subspecies of Red-crowned Parakeets *C. novaezelandiae* (Nei's $D = 0.008$). Triggs and Daugherty (1996) also concluded that the Orange-fronted Parakeet

was the sister taxon of Yellow-crowned Parakeet. However, Taylor (1998) again disputed that there are any significant morphometric differences or reproductive isolation between sympatric Orange-fronted and Yellow-crowned Parakeets from Lake Sumner Forest Park, New Zealand.

History of Forbes' Parakeet

Forbes' Parakeet was first described by Rothschild (1893) as a distinct species *C. forbesi* but was later relegated to subspecies *C. a. forbesi* of the Yellow-crowned Parakeet *C. auriceps* by Oliver (1930) without justification. Morphological studies showed Forbes' Parakeet to be larger than Yellow-crowned Parakeets (Fleming 1939, Nixon 1982). Markedly differing vocal repertoires were also observed by Pickard (1990). Based on their allozyme data, Triggs and Daugherty (1996) found that Forbes' Parakeet is genetically very divergent (Nei's $D = 0.05$) from all Yellow-crowned Parakeets and should be considered a separate species.

Experimental strategy

In this paper, we test existing taxonomic hypotheses for two species of the genus *Cyanoramphus* based on detailed analyses of mitochondrial cytochrome *b* gene (1,140 nt) and control region (1,577–1,582 nt) DNA sequences. The rapid rate of evolution, almost complete lack of recombination and predominantly maternal mode of inheritance of mitochondrial DNA (Gyllensten *et al.* 1991, Lansman *et al.* 1983) make reconstructing the phylogenetic history of mitochondrial genes simpler than for nuclear markers. We also carried out field observations in the south branch of the Hurunui Valley (1996–1999) to look for mixed pairs of Yellow-crowned and Orange-fronted Parakeets as a direct test for interbreeding between these two types. The hypotheses tested are as follows.

Hypothesis 1

That the Orange-fronted Parakeet is a colour morph of sympatric Yellow-crowned Parakeet (Holyoak 1974, Taylor *et al.* 1986, Taylor 1998) and does not itself constitute a separate species. This view would be supported if, and only if, (a) there is no diagnostic genetic differentiation between the two forms and (b) mixed breeding pairs occur in the wild. Contrary findings would support specific recognition of the Orange-fronted Parakeet as *C. malherbi* under the phylogenetic, biological, cohesion and recognition concepts of species.

Hypothesis 2

That the Orange-fronted Parakeet is the sister taxon of Yellow-crowned Parakeet (Triggs and Daugherty 1996). Finding that they are the sister group to another clade of *Cyanoramphus* parakeets based on analysis of DNA sequence would falsify this hypothesis and strongly support its specific status based on the phylogenetic species concept.

Hypothesis 3

That Forbes' Parakeet is sufficiently genetically distinct from Yellow-crowned Parakeet to merit full species recognition under the phylogenetic species concept, as proposed by Triggs and Daugherty (1996).

Methods

Seventeen individuals of *Cyanoramphus* parakeets were examined in this study. They represent nine different populations and six taxa (Table 2). All biological samples were in the form of frozen red blood cells and up to 2,722 nt of DNA sequence data were collected per individual for phylogenetic analyses. The cytochrome *b* gene was sequenced in nine individuals as a preliminary study in order to determine the suitability of this target for resolving the phylogenetics of the taxa in question. Based on these results, the control region was judged to be a superior locus for this purpose. Detailed molecular phylogenetic analysis of all 17 individuals was then carried out using the control region as a target.

DNA extraction

For DNA preparations, 10 µl of red blood cells were added to 500 µl of RSB buffer (10 mM Tris HCl pH 7.4, 10 mM NaCl, 25 mM ethylenediamine tetraacetic acid (EDTA), and lysed by the addition of sodium dodecyl sulphate (SDS) and proteinase K (Life Technologies). The digest was extracted with phenol/chloroform mixtures, and washed in a microconcentrator (Amicon) with sterile micro-filtered water and concentrated to 100 µl final volume in TE pH 8.0 solution (10 mM Tris pH 8.0, 0.1 mM EDTA pH 8.0) and stored at -80°C until required (Sambrook *et al.* 1989).

Polymerase chain reaction

Cytochrome *b* Two partially overlapping segments 477 and 932 nt of the cytochrome *b* gene were amplified via the polymerase chain reaction (PCR) using primer pairs L14827 (Helm-Bychowski and Cracraft 1993)/H15305 (G.K.C., unpublished) and L15132 (modified from primer CB II, Dawson 1992)/H16065 (modified from primer no. 15915R, Irwin *et al.* 1991) respectively (Table 3). Together, the two segments cover the complete 1,140 nt cytochrome *b* gene and part of the tRNA^{Thr} and ND5 genes flanking cytochrome *b* (Figure 2). Additional short (317 nt) DNA sequences between the primer pair L14987/H15305 were obtained for individuals FT3304, FT3305, FT3308, and FT3315 (Tables 4a, b). The cytochrome *b* gene was PCR amplified using aliquots (4 µl) of each purified DNA solution as template in a 100 µl reaction mixture: 0.5 µM each primer, 125 µM deoxynucleotide triphosphates (dNTPs), 5 units *Taq* DNA polymerase (Life Technologies), 1.5 mM MgCl₂, 10 µl 10x *Taq* buffer, made up to 100 µl with sterile microfiltered water and overlaid with mineral oil. Thermal cycling was performed in a Perkin-Elmer model 480 machine, with an initial denaturation step at 95 °C for 3 mins, followed by 35 cycles of denaturation at 95 °C for 40 s,

Table 2. Catalogue of *Cyanoramphus* parakeet samples analysed in this study

Common name	Taxon as recognized in this study	Collection number(s)	Locality
Antipodes Island (Green) Parakeet	<i>C. unicolor</i>	CD1130	Antipodes Island
Red-crowned Parakeet	<i>C. n. novaezealandiae</i>	CD1212 *FT1016 *CD2035	Nga Manu Wildlife Sanctuary, southern North Island (Captive) Little Barrier Island, northern North Island Poor Knights Island, northern North Island
Chatham Island Red-crowned Parakeet	<i>C. n. chathamensis</i>	PK23 *CD1838	Mangere Island, Chatham Islands; east of N.Z. mainland South East Island, Chatham Islands; east of N.Z. mainland
Forbes' Parakeet	<i>C. forbesi</i>	CD1814	Mangere Island, Chatham Islands; east of N.Z. mainland
Yellow-crowned Parakeet	<i>C. auriceps</i>	*FT1029 CD1878 WG168 FT3303/ 3304/3305 FT3308	Little Barrier Island, northern North Island Chetwode Islands, northern South Island Eglinton Valley, Fiordland National Park, South Island South branch Hurunui Valley, Lake Sumner Forest Park, South Island Hawdon Valley, Arthur's Pass National Park, South Island
Orange-fronted Parakeet	<i>C. malherbi</i>	FT3314/ 3315/3316	South branch Hurunui Valley, Lake Sumner Forest Park, South Island

All blood samples and DNA extracts are held in the Institute for Molecular Systematics, School of Biological Sciences, Victoria University of Wellington, New Zealand. Asterisks indicate specimens excluded from cytochrome *b* analysis. Collection numbers refer to individual parakeets. Individual CD1212 is morphologically a Red-crowned Parakeet but may have a hybrid origin (Red-crowned × Yellow-crowned Parakeets). The genealogical history of individual CD1212 is unknown, therefore in the context of this study it is referred to as the "captive CD1212 Red-crowned Parakeet".

annealing at 55 °C for 40 s and extension at 72 °C for 60 s and terminated by a final extension step for 10 mins at 72 °C.

Control region A 2.5 kb segment of the *Cyanoramphus* mitochondrial genome was amplified using primer pair L16518/H1800 (Table 3) for all individuals (Table 2). This segment is flanked by the 3' end of the ND6 gene and 5' end of 12S rRNA gene and encompasses the entire control region, plus tRNA^{Phe} and tRNA^{Glu}. To amplify the 2.5 kb segment, PCR were performed as above using 8 µl of each purified DNA solution using a reaction mix: 0.5 µM each primer, 100 µM dNTPs, 5.25 units ExpandTM high fidelity (HF) DNA polymerase enzyme mix (Roche), 10 µl 10× ExpandTM HF buffer with 15 mM MgCl₂ (Roche), made up to 100 µl with sterile microfiltered water. A modified amplification protocol was used consisting of denaturation at 95 °C for 3 mins, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s and extension at 68 °C for 2

Table 3. Primers used for polymerase chain reaction (PCR) amplification and cycle sequencing

Primer name	Primer sequence (5' to 3')
L70-90 (a)	GTA CGT CAC GGG CTC TTT TAG TCC
L70-90 (b)	GTC ACG GGC TCT TTT AGT CCT TTA TGG
L90-110	AAC TTC ACG CCC TCG GAT AGA ATA
L531	TGC TCT TTT GTG CCT CTG GTT CCT C
L650	AGC GCC TTG TCT CTG TTG G
L14827	CCA CAC TCC ACA CAG GCC TAA TTA A
L14987	CCC CTC AAA TAT CTC CAT ATG ATG
L15132	CGA ACC GTA CAA TAC GGA TGG YTA ATC
L15643	CTA CCC TAG CCC TCT TCT CAC CCA ACC TAC
L16518	GAC GGG AAT AAA CAA AAA CCA CCA ACA
H100-200	GAC TGA AGT GAG ACT ATT CCT TGA GAC
H519	ATG CGA CTT GAC CGA GGA ACC AGA GG
H646	GGC TAC CCA GAG AAA AAA AAC CAA C
H1529	TGG CTG GCA CAA GAT TTA CCG
H1800	CCC CCG TTT GTG CTC GTA GTT CTC
H15163	GGC GAT GTG GAG GTC GAT GCA GAT GAA GAA
H15305	AAA CTG CAG CCC CTC AGA ATG ATA TTT
H15706	GGC AAA TAG GAA RTA TCA TTC
H15977	AGA TGA TGG GGA ATA GGA TTA GGA TGA
H16065	TCA TCT CCG GTT TAC AAG AC

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 and Morais 1990). Some primers used do not align well with the chicken mitochondrial DNA sequence, thus a range of nucleotide positions is given instead of absolute positions.

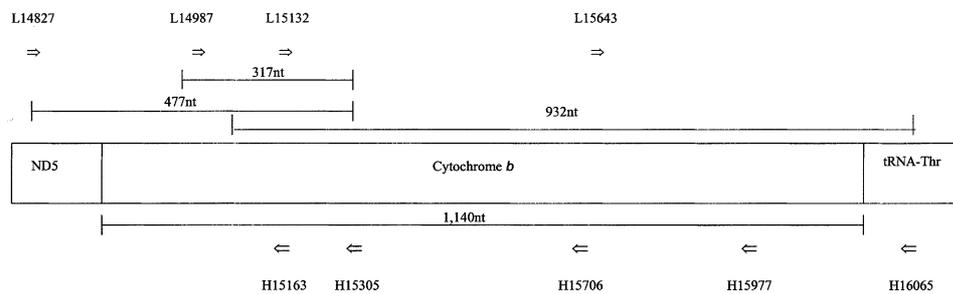


Figure 2. Strategy for polymerase chain reaction (PCR) amplification and cycle sequencing of the cytochrome *b* genes. Arrows denote primers and their orientation. The numbers refer to their positions relative to the chicken mitochondrial DNA sequence (Desjardins and Morais 1990) and letters to the "light" and "heavy" strands of the mitochondrial DNA (Table 3). We have confirmed that the gene order for *Cyanoramphus* matches the chicken gene order by sequencing of long-PCR (5.6 kb) product (data not included in this study). Conserved sequence blocks C, D and CSB-1 (see Baker and Marshall 1997, Mindell *et al.* 1998) were also found within the control region sequences obtained. The bars above show the location of fragments amplified and that below, the overlapping region of sequence included in analysis. The sizes of gene targets are not drawn to scale.

Table 4a. DNA sequence divergence of mitochondrial cytochrome *b* gene and control region between *Cyanoramphus* parakeet species: table of transversion substitutions

	Orange-fronted			Yellow-crowned							Red-crowned				
	FT 3314 Hurunui	FT3315 Hurunui	FT3316 Hurunui	FT3303 Hurunui	FT3304 Hurunui	FT3305 Hurunui	FT3308 Hawdon	WG168 Eglinton	CD1878 Chet- wodes	FT1029 Little Barrier	CD2035 Poor Knights	CD1838 South East	FT1016 Little Barrier	PK23 Mangere	CD1212 Cap- tive
FT3314		0/1	0/1	42/2	40/3	40/4	43/3	40/2	47/2	43/2	34/2	33/0	32/1	31/2	41/6
FT3315	0/0		0/0	42/1	40/4	40/5	43/2	40/1	47/1	43/1	34/1	33/1	32/0	31/1	41/7
FT3316	0/0	0/0		42/1	40/4	40/5	43/2	40/1	47/1	43/1	34/1	33/1	32/0	31/1	41/7
FT3303	4/0	2/0	4/0		12/3	12/4	11/1	10/0	15/0	11/0	44/2	47/2	46/1	45/2	47/8
FT3304	2/0	2/0	2/0	0/0		0/3	10/4	7/3	10/3	11/3	43/5	44/3	43/4	42/5	47/9
FT3305	2/0	2/0	2/0	0/0	0/0		10/5	7/4	10/4	11/4	43/6	44/4	43/5	42/6	47/10
FT3308	1/0	1/0	1/0	1/0	1/0	1/0		7/1	12/1	12/1	45/3	46/3	43/2	44/3	48/9
WG168	4/0	1/0	4/0	2/0	1/0	1/0	0/0		9/0	9/0	44/2	45/2	44/1	43/2	45/8
CD1878	4/0	1/0	4/0	2/0	1/0	1/0	0/0	2/0		14/0	49/2	50/2	49/1	48/2	52/8
FT1029	—	—	—	—	—	—	—	—	—	—	47/2	48/2	45/1	46/2	50/8
CD2035	—	—	—	—	—	—	—	—	—	—	—	19/2	15/1	17/2	33/8
CD1838	—	—	—	—	—	—	—	—	—	—	—	—	18/1	2/2	40/6
FT1016	—	—	—	—	—	—	—	—	—	—	—	—	—	16/1	35/7
PK23	2/0	1/0	2/0	2/0	1/0	1/0	0/0	2/0	2/0	—	—	—	—	—	38/8
CD1212	4/0	2/0	4/0	4/0	2/0	2/0	1/0	4/0	4/0	—	—	—	—	2/0	—
CD1814	20/0	8/0	20/0	18/0	8/0	8/0	7/0	18/0	18/0	—	—	—	—	18/0	20/0
CD1130	6/0	1/0	6/0	6/0	1/0	1/0	2/0	8/0	8/0	—	—	—	—	6/0	8/0

Data for cytochrome *b* are presented below the diagonal and for control region above the diagonal. The values indicate numbers of transition/transversion (ts/tv) for each comparison made. **Values in bold** refer to comparisons made for 317 nt of the cytochrome *b* gene only. Complete cytochrome *b* gene: 1,140 nt. Complete mitochondrial control region: 1,584 nt.

mins. The last 25 cycles had a cumulative increase of extension time of 20 s/cycle. A final extension step of 68 °C was carried out for 7 mins after the completion of 35 cycles.

PCR product purification and quantitation

Double-stranded DNA amplification products were purified on 1% low-melting agarose gels (FMC Bioproducts) and extracted with a Biorad Prep-A-Gene DNA purification kit. The concentration of purified products was estimated visually following electrophoresis, by comparison with the High DNA Mass™ ladder (Life Technologies).

DNA sequencing

Cycle DNA sequencing was performed on purified double-stranded DNA products according to the ABI Prism® Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (RR) protocol (Perkin-Elmer 1998) using 40–60 ng template. Sequencing primers used to sequence the cytochrome *b* gene include the original PCR primers plus H15163 (modified from CB I, Dawson 1992), L14987 (modified from primer no. 14841, Kocher *et al.* 1989), H15977, L15643 (this study) and H15706 (modified from primer H15547, Edwards *et al.* 1991). Primers used to sequence the control region segment were L70–90 (a and b), L90–110, L531, L650, L16518, H100–200, H519, H646 and H1529 (this study; see Table 3). The positions of PCR and sequencing primers are shown relative to the targets and the genes flanking them in Figures 2 and 3. Fluorescently labelled products from cycle sequencing reactions were purified and analysed on an ABI Model 377 Prism® automated DNA sequencer (Perkin-Elmer) according to the protocol mentioned above.

DNA sequence and phylogenetic analyses

Sequences were obtained from both light and heavy strands of each target region and combined to produce unambiguous contiguous consensus sequence files with DNASTAR's Lasergene 99 data acquisition and analysis package (Anon 1997). Consensus DNA sequences for each individual were aligned with XESEE 3.2 program (Cabot 1998). Sequence statistics were produced and compared using MEGA 1.01 (Kumar *et al.* 1993).

All phylogenetic analyses were performed using the heuristic algorithm in PAUP* 4.0 beta version (Swofford 1998). A maximum parsimony (MP) tree was constructed based on all parsimony informative characters without weighting. Deletions in the sequence data are treated as a fifth character state. Maximum likelihood (ML) and minimum evolution (ME) analyses were carried out based on the General Time Reversible substitution model of Rodríguez *et al.* (1990) with gamma approximation ($\alpha = 0.17$). Bootstrap resampling (Felsenstein 1985) was carried out to provide an assessment of support for *Cyanoramphus* clades identified from control region sequences (MP 5,000 replicates, ML 300 replicates, ME 1,000 replicates).

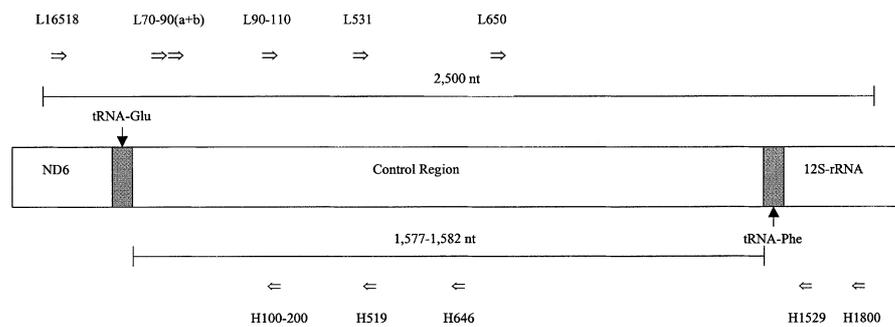


Figure 3. Strategy for PCR amplification and cycle sequencing of the mitochondrial control region. Arrows denote primers and their orientation. The numbers refer to their positions relative to the chicken mitochondrial DNA sequence (Desjardins and Morais 1990) and letters to the "light" and "heavy" strands of the mitochondrial DNA (Table 3). We have confirmed that the gene order for *Cyanoramphus* matches the chicken gene order by sequencing of long-PCR (5.6 kb) product (data not included in this study). Conserved sequence blocks C, D and CSB-1 (see Baker and Marshall 1997, Mindell *et al.* 1998) were also found within the control region sequences obtained. The bar above the genes shows the location of amplified fragment and that below, the region of sequence included in analysis. The sizes of gene targets are not drawn to scale.

Parakeet pairing data collection

As part of a larger study into the ecological relationships between sympatric Orange-fronted and Yellow-crowned Parakeets in the south branch of the Hurunui Valley (North Canterbury), the occurrence of mixed "pairs" was surveyed in an area of beech *Nothofagus* spp. forest (7.5×0.5 km) on the flat section of the valley floor. This constituted approximately 20% (by area) of the total nesting habitat available to both Yellow-crowned and Orange-fronted Parakeets within the valley. The present estimate of wild Orange-fronted Parakeet numbers indicates less than 500 individuals (Kearvell 1999). They are currently known only to persist in two locations, the south branch of the Hurunui River and the Hawdon River valleys. Recent transect counts have shown that Orange-fronted Parakeets are encountered 10 times more frequently in the south branch of the Hurunui Valley than in the Hawdon Valley (Kearvell 2000). The area surveyed in the former location thus covers a significant proportion of the known Orange-fronted Parakeet population range. Due to the large area involved, the survey site was arbitrarily divided into two approximately equal sections and each surveyed on consecutive days. If the weather was poor, the other section was counted the following day. Counts were carried out during spring and summer, between 13 November 1998 and 24 February 1999, covering 48 days of survey. The forest in this area was homogeneous. This was tested using the forest survey "Point-Centre-Quarter" technique (Cottam and Curtis 1956, Greig-Smith 1964).

All positively identified parakeets were given a map coordinate. They were then assessed for pair status. The criterion for assessing whether or not two birds were a pair was designed to identify even the most tentative of examples and thus identify any possible chances that the two species were forming mixed breeding pairs rather than pairing in a strictly assortative manner. The primary

Table 5. Number of confirmed parakeet pairs in the survey area, south branch Hurunui Valley, New Zealand during the 1998/1999 summer season

Season	N	Number of confirmed unique pairs	Mixed pairs	Confirmed nests
Orange-fronted Parakeet	516	32	0	17
Yellow-crowned Parakeet	570	26	0	13
Totals	1086	58	0	30

N is the total number of birds recorded over all occasions within field season. $N = (\text{number of confirmed unique pairs} \times 2) + \text{solitary birds which have been positively identified either as a Yellow-crowned or Orange-fronted Parakeet} + (\text{number of pairs with coordinates which overlapped another pair} \times 2)$. Mixed pairs is the total number of confirmed interspecific pairs identified. Detailed count data are only given for the 1998/1999 season, using the coordinate method (described in Methods) to avoid counting pairs more than once. Preliminary surveys recorded 9 pairs of Orange-fronted Parakeet ($N = 67$) and 27 pairs of Yellow-crowned Parakeet ($N = 161$) in the 1996/1997 season. The corresponding values were 18 pairs of Orange-fronted ($N = 65$) and 15 pairs of Yellow-crowned Parakeets ($N = 78$) for the 1997/1998 season. No mixed pairs were recorded in either year. However, since coordinates were not recorded in 1996/1997 and 1997/1998 seasons we cannot be certain that some pairs were not counted more than once. In 1998/1999, for comparison, 186 pairs of Orange-fronted and 140 pairs of Yellow-crowned Parakeets were originally recorded, but these were reduced to 32 and 26 confirmed unique pairs after coordinate analysis.

criterion for a "pair" was that the two birds in question appeared to be associating together without influence from a third bird (unless one bird was either a fledgling or a nestling or the three birds were all in a situation of aggressive interspecific display) and their behaviour was collaborative (i.e. preening, courting, nest hole inspection, mating, feeding fledglings/nestlings, egg incubation or simply sitting on a branch), not aggressive. Each pair was observed for as long as possible in order to verify their specific status and confirm non-aggressive behaviour. To avoid multiple counting of the same pair, no new pair record was accepted unless it was outside a 100 m radius from the nearest pair contact and was encountered after more than one minute from the last positive pair record. New Zealand parakeets have non-exclusive home ranges (Elliott *et al.* 1996, Greene 1998) and perform most of their breeding behaviour less than 50 m from their actual/prospective nest site.

The survey was also carried out in the Hurunui Valley during the summer seasons of 1996/1997, 1997/1998. It is important to note that repeat counting of pairs may have occurred during these two seasons because map coordinates were not recorded during these years but were in the 1998/1999 season as described earlier. All data obtained are presented in Table 5.

Results

The two segments of the mitochondrial cytochrome *b* gene and the entire control region were readily amplified for most samples. For some samples, optimization of PCR conditions (lowering of annealing temperature to 54 °C or increasing cycle numbers to a total of 40) was required before the desired level of target

amplification was achieved. In PCR catalysed amplifications, the expected 477/932 nt and 2.5 kb targets were consistently amplified. Slight variation in product size was observed for the 2.5 kb targets. This is expected, since it includes the mitochondrial control region, which is characterized by its high variability, the occurrence of expanding repeat units, insertions and deletions. Negative control PCR reactions showed no amplification products in any case. All DNA sequence electrophoretograms could be read unambiguously, and data representing almost all nucleotide positions were confirmed by sequencing from both directions or from at least two different primers in the same direction. The acquired DNA sequences for each target for each individual were combined to obtain consensus sequences and these were aligned and compared with consensus sequences of other *Cyanoramphus* individuals (Figures 4 and 5). The *Cyanoramphus* cytochrome *b* sequences aligned well with those published for other *Psittaciformes* (Birt *et al.* 1992, Leeton *et al.* 1994, Miyaki *et al.* 1998) and for chicken (Desjardins and Morais 1990), alignments are not shown in this report, and form the basis of a wider taxonomic study of the placement of the *Cyanoramphus* genus within *Psittaciformes* (Boon *et al.* unpubl.). Control region sequences could only be aligned with the chicken sequences at highly conserved regions (alignments not shown) and no other parrot mitochondrial control region sequences were available for comparison. The GenBank accession numbers for sequences reported in this paper are AF218735–AF218764.

Direct sequencing of PCR products from mitochondrial DNA templates may sometimes yield data that include a mixture of both authentic mitochondrial and nuclear copies of mitochondrial gene sequences leading to single nucleotide ambiguities or more serious artifacts (Smith *et al.* 1992, Lopez *et al.* 1994, Sorenson and Fleischer 1996). During our analyses, we examined all of the experimental data for molecular signatures characteristic of mitochondrial DNA genes and their nuclear homologues. Such characteristics include codon-specific pattern of substitutions, lack of stop codons, insertions/deletions, frameshifts or chemically non-conservative amino acid changes in coding regions, high transition:transversion ratio – especially of comparisons between closely related taxa, characteristic among site rate variation and nucleotide frequencies, identification of conserved sequence blocks (e.g. D and C box, CSB-1 in mitochondrial control region) and the use of highly specific as opposed to “universal” sequencing and PCR primers (Mindell *et al.* 1998, Norman *et al.* 1998, Baker and Marshall 1997 and Zhang and Hewitt 1996). In summary, we were satisfied by the above observations that we had obtained authentic mitochondrial DNA sequences in each case and not nuclear insertions of mitochondrial DNA genes or “Numts” (see Quinn 1997 for further general discussion of evidence and comments regarding “Numts”). We have also confirmed that the gene order for *Cyanoramphus* parakeets matches the chicken *Gallus gallus* (Mindell *et al.* 1998, Desjardins and Morais 1990) thus adding a parrot taxon to the group of birds with that particular gene configuration. This was done by sequencing a 5.6-kb-long PCR product (data not included). Supporting this conclusion, PCR primers (L16518 in ND6 gene; H1800 in 12S rRNA gene), which were designed to amplify the mitochondrial control region produced sequences that had molecular signatures (e.g. motifs C, D and CSB-1, see Mindell *et al.*, 1998) characteristic of the target concerned. The data

presented in this paper include the total number of transitions/transversions (ts/tv) and uncorrected percentage divergence between different taxa compared (Tables 4a, b).

Sequence variation and population differentiation

Cytochrome b A total of 28 variable sites were identified among the cytochrome *b* sequences for the nine individuals examined (Figure 4) with 8 at the first codon position, 0 at the second and 20 at the third. No insertions or deletions were detected, and all nucleotide substitutions were transitions, suggesting an exceptionally high ts/tv ratio (> 20:1). This pattern is highly characteristic of recently diverged mitochondrial DNA haplotypes (Quinn 1997, Moritz et al. 1987).

The cytochrome *b* sequences observed are identical for all three Orange-fronted individuals examined whereas intraspecific comparisons within Yellow-crowned Parakeets and Red-crowned Parakeets display sequence variation of 0.18%. All percentages presented in the Results and Discussion of this paper refer to the complete cytochrome *b*. Interspecific comparisons of Orange-fronted and Yellow-crowned Parakeets show a higher level of sequence divergence, around 0.35%, whereas comparisons of Orange-fronted Parakeet with Red-crowned Parakeet cytochrome *b* sequences show a range of difference from 0.18 to 0.35%. Interspecific comparisons of the Antipodes Island Parakeet *C. unicolor* with all other species (excluding Forbes' Parakeet) ranged from 0.53 to 0.70%, being much higher than other comparisons (Table 4b). Forbes' Parakeet displayed the highest level of divergence from all other species with a range of 1.58 to 1.70%, well beyond any other interspecific comparison.

Also unique to the Forbes' Parakeet nucleotide sequence were two transitions giving rise to inferred amino acid substitutions at positions 43 (ala for thr) and 307 (leu for phe). Using the Orange-fronted Parakeet cytochrome *b* protein sequence as reference, a change at amino acid position 53 (ala for thr) was

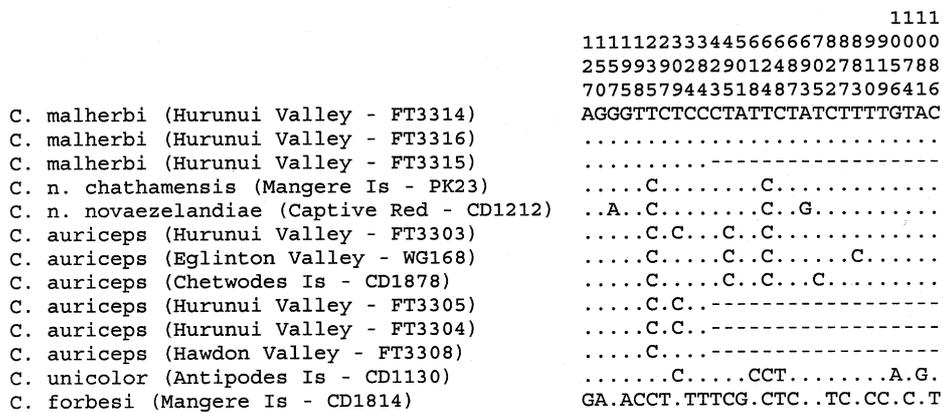


Figure 4. Variable sites of aligned cytochrome *b* sequences of 17 *Cyanoramphus* individuals. Numbers above the sequences indicate the position of the variable site corresponding to positions 1-1,140 of aligned sequences. Dashes indicate missing data.

observed for the Antipodes Island Parakeet and at position 361 (thr for ala) for the captive Red-crowned Parakeet.

Control region Of 1,584 sites compared in the *Cyanoramphus* mitochondrial control region 217 were variable. These provided 91 parsimony informative sites for cladistic analysis. Genetic distances ranging from 0 to 7.87% (uncorrected) were observed among the 17 individuals examined. Within species, control region sequences from Orange-fronted Parakeets are very homogenous, with individuals differing at between 0.00 and 0.06% of sites. The DNA sequences from Yellow-crowned Parakeets displayed higher intraspecific divergence, ranging from 0.19 to 1.01% between individuals. The highest level of intraspecific divergence observed was between individual Red-crowned Parakeet sequences. Values ranged from 0.25% in comparisons of DNA sequences within Chatham Island Red-crowned Parakeet populations to 2.92% in comparisons between Chatham Island Red-crowned Parakeet populations and the captive CD1212 Red-crowned Parakeet (see Table 2 for guide on nomenclature). The low intraspecific divergence for the Orange-fronted Parakeet could be due in part to the limited geographical range of this species.

The control region sequences from the Orange-fronted Parakeets are most similar to those from Little Barrier Island and Mangere Island Red-crowned Parakeets (2.03% divergence). This level of genetic differentiation is lower than comparisons between mitochondrial control region sequences from Orange-fronted Parakeet and sympatric Yellow-crowned Parakeet individuals (2.72–2.85%). The levels of genetic divergence between Red and Yellow-crowned Parakeets were close to this and ranged from 2.85 to 3.80%. The sequences from Antipodes Island Parakeet displayed consistently high levels of interspecific divergence paralleling those shown earlier by the cytochrome *b* data and ranged from 3.49 to 4.25%. Again, Forbes' Parakeet is well differentiated from all other *Cyanoramphus* species examined in this study. Genetic distances between the single Forbes' Parakeet sequence reported here and others is between 7.49 and 7.80%; i.e. much greater than the values seen in other interspecific comparisons between *Cyanoramphus* taxa. We note that the sequence presented is representative of the major Forbes' Parakeet mitochondrial DNA haplogroup and have since obtained identical or very closely related (0–17 nt substitutions out of 1573–1577 nt compared) sequences for five further individuals (Boon *et al.* unpubl.).

Field observations

A total of 1,086 individual parakeets were positively identified during the 1998/1999 survey period. From these, 106 confirmed pair contacts were obtained, which was eventually reduced to 58 unique pairs after analysis of coordinates. No mixed Yellow-crowned/Orange-fronted Parakeet pair was encountered. Sympatric Orange-fronted and Yellow-crowned Parakeets, in the Hurunui Valley, appear to court, mate and nest strictly assortatively (Table 5). During the collection of these data, further pairs were observed undertaking feeding and maintenance behaviour (see legend, Table 5). No mixed pairs were observed within these categories. Yellow-crowned Parakeets have been recorded feeding at considerable distances from nest sites (Elliott *et al.* 1996), which makes repeat

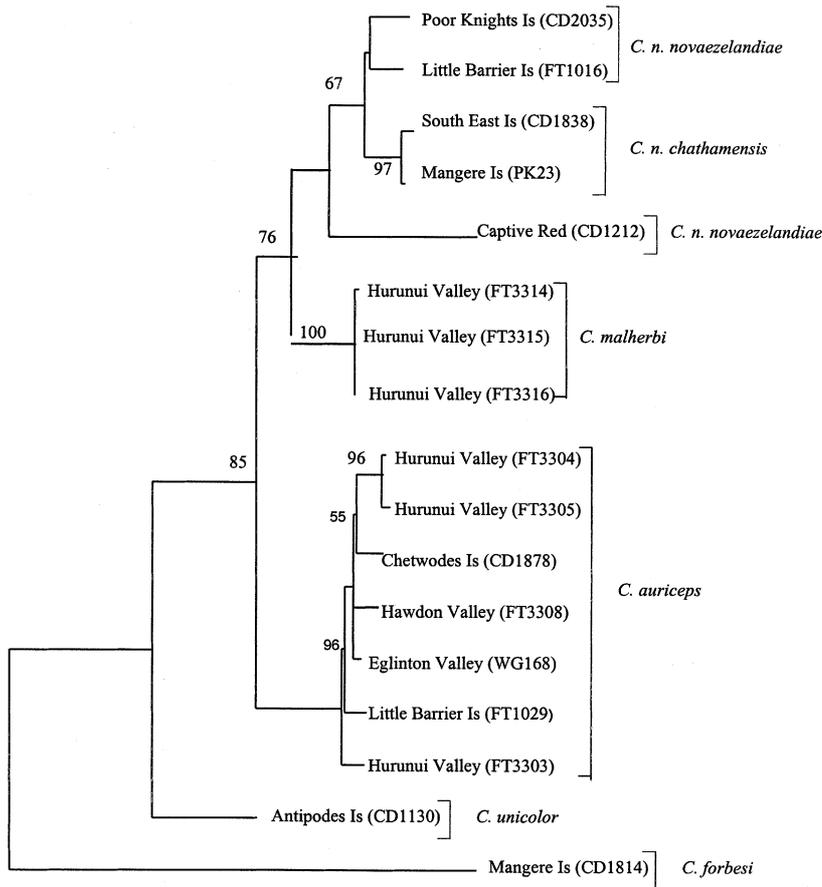


Figure 7. Maximum likelihood tree based on control region data for 17 *Cyanoramphus* individuals. *C. forbesi* is used as outgroup. Bootstrap values greater than 50% are indicated at nodes (300 replicates). The General Time Reversible substitution model (Rodríguez *et al.* 1990) and gamma approximation ($\alpha = 0.17$) of variable sites were used.

together in a distinct group and they are clearly the sister taxon of the Red-crowned Parakeet rather than Yellow-crowned Parakeet. All of the Yellow-crowned Parakeet individuals group into a single fully supported clade with very little population structure. The Chetwodes Island Yellow-crowned Parakeet appears to be the sister taxa of the Hurunui Valley cluster, in both the Minimum Evolution and Maximum Likelihood trees, but clusters with the Eglinton Valley individual in the Maximum Parsimony tree. The three well-supported and entirely distinct clades comprise Yellow-crowned, Orange-fronted and Red-crowned birds with Antipodes Island Parakeet being a clear outlier and Forbes' Parakeet as the basal taxon. In all analyses, Forbes' Parakeet is clearly genetically differentiated from Yellow-crowned Parakeet. The genetic distances between Forbes' Parakeet and either Red or Yellow-crowned Parakeets are three to four times greater than between Red and Yellow-crowned Parakeets.

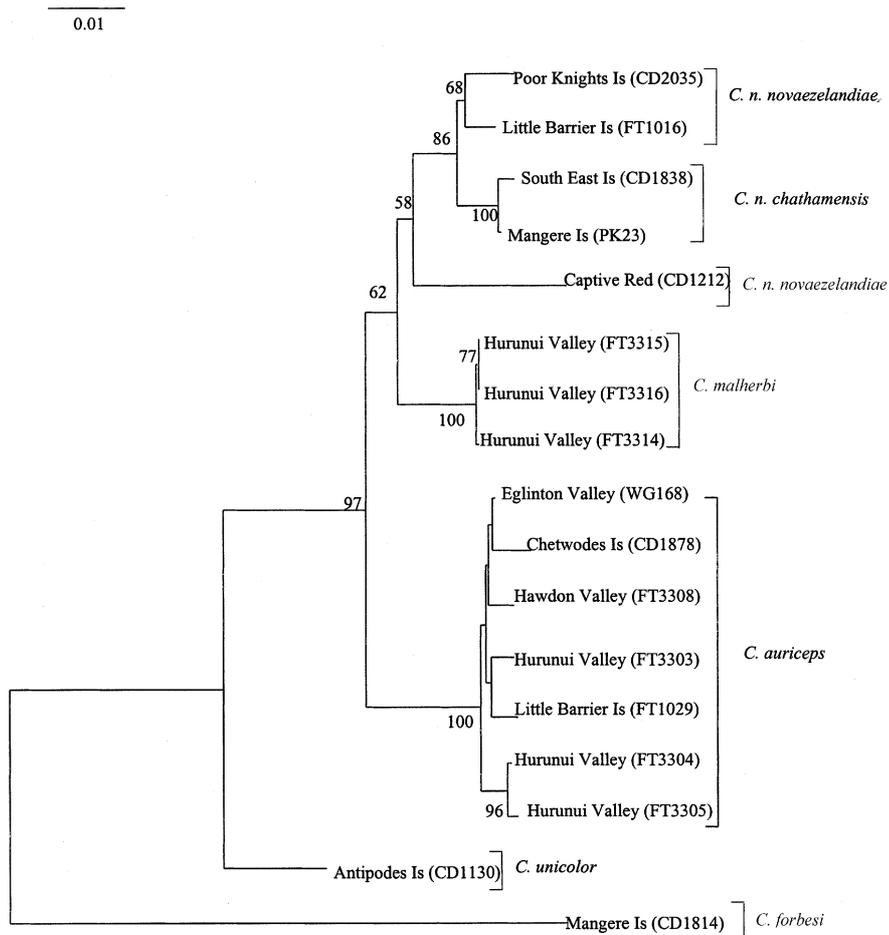


Figure 8. Minimum evolution tree based on control region data for 17 *Cyanoramphus* individuals. *C. forbesi* is used as outgroup. The General Time Reversible distance (Rodríguez et al. 1990) was used for the minimum evolution tree above ($\alpha = 0.17$). Bootstrap values (1,000 replicates) for branches are indicated by corresponding arrows.

Discussion

Species concepts

Determination of species status should be viewed as testing a hypothesis (Simpson 1961, Baum and Shaw 1995) and should be based on the best interpretation of all relevant available evidence (Graybeal 1995). In this study we apply four species concepts to our data, the Biological Species Concept (BSC; Mayr 1942, 1970), the Phylogenetic Species Concept (PSC; Cracraft 1983, 1997), the Recognition Species Concept (RSC; Paterson 1985) and the Cohesion Species Concept (CSC; Templeton 1989).

A "biological species" is a group of interbreeding individuals in a natural

population that is reproductively isolated from other such groups. A "phylogenetic species" can be uniquely diagnosed and has a parental pattern of ancestry and descent. The latter criterion often refers to the monophyly of the group in question. The RSC defines a species as "the most inclusive population of individual biparental organisms which share a common fertilization system". Lastly, a "cohesion species" is the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms. Our evidence includes identification of separate gene pools, diagnosability and monophyly of each species and field evidence on mate choice. The genetic and field data obtained satisfy practical criteria under all of the above species concepts for both Orange-fronted and Forbes' Parakeets.

Taxonomic status

Orange-fronted Parakeet We have found significantly large, distinct, consistent and apparently fixed genetic differences between Orange-fronted Parakeets and the sympatric population of Yellow-crowned Parakeets in the Hurunui Valley. This demonstrates exclusivity of the Orange-fronted Parakeet mitochondrial gene pool. Both synapomorphic (parsimony informative) and autapomorphic (private) characters were observed to differ between Yellow-crowned and Orange-fronted Parakeets. These are diagnostic for species and certainly reveal patterns of ancestry and descent. Based on the level of genetic differentiation and cladistic pattern observed, the Orange-fronted Parakeet forms a readily diagnosable and monophyletic taxon fulfilling the PSC. Based on percentage divergence between mitochondrial control region nucleotide sequences (Tables 4a, b), our data place Orange-fronted Parakeet and sympatric Yellow-crowned Parakeet well beyond the level of interspecific genetic divergence observed between other interspecific comparisons of accepted *Cyanoramphus* species (Figure 8). Very well-supported (bootstrap value = 91%) parsimony analysis placed Red-crowned Parakeet as the sister species of Orange-fronted Parakeet. This finding is contrary to current classifications. We therefore reject Hypothesis 2, thus supporting further the specific status of the Orange-fronted Parakeet.

Field observations in the Hurunui Valley, where the largest known population of Orange-fronted Parakeet exists, indicate strong assortative mating (Table 5). The absence of mixed (Yellow × Orange) nesting pairs therefore fulfils the practical criterion of reproductive isolation under the BSC as advanced by Mayr (1970). Strongly assortative mating implies separate mate recognition systems for Orange-fronted and Yellow-crowned Parakeets, thereby satisfying the RSC of Paterson (1985). The above observations corroborate the exclusivity of the mitochondrial gene pools displayed by Orange-fronted and Yellow-crowned birds respectively. This indicates the existence of cohesion mechanisms within each of the "species", accommodating the key criterion of the CSC according to Templeton (1989).

These findings dispute Taylor's (1998) claim that there is no evidence for assortative mating in the two taxa. However, when non-specific mates are rare, many *Cyanoramphus* species seem to be able to hybridize successfully with any close congener, as observed on Mangere Island (Forbes' × Red-crowned

Parakeets) and the Auckland Islands (Yellow × Red-crowned Parakeets). Taylor *et al.*'s (1986) captive breeding experiments showed the viability of Orange-fronted × Yellow-crowned hybrids and an apparently simple genetic basis for plumage characters. In contrast, Grant and Grant (1992, 1997) reviewed the hybridization and speciation of birds and found that the late development of post-mating isolating factors during avian speciation results in 9.2% of presently described bird species being able to produce hybrid offspring in congeneric crosses. Moreover, the finding of a simple or complex genetic basis for any single characteristic of a bird does not fulfil the lead criteria of any species concept. This negates the idea that Taylor *et al.*'s (1986) captive cross breeding test is in any way applicable for species diagnosis in *Cyanoramphus* (or any other avian genus for that matter). There is always a possibility that gene flow may have occurred as a result of strictly directional hybridization between male Yellow-crowned and female Orange-fronted Parakeets. This would be undetected by mitochondrial DNA analysis and further work is presently under way (Chan *et al.* unpubl.) to test this hypothesis using nuclear microsatellite markers. For the present moment, this seems an unlikely prospect since there is no evidence of unidirectional hybridization in aviary crosses (Taylor *et al.* 1986). The fact that Yellow-crowned and Orange-fronted Parakeets are not sister taxa (Figures 6–8) makes it unlikely that they are conspecific. In consideration of the above molecular genetic and field data, we believe that the Orange-fronted Parakeet represents a genuinely distinct species, hence we reject the colour-morph hypothesis (Hypothesis 1) as proposed by Holyoak (1974), Taylor *et al.* (1986) and Taylor (1998) and classify it as *Cyanoramphus mallherbi* after Triggs and Daugherty (1996) and Souance (1857).

Forbes' Parakeet The taxonomic rank of Forbes' Parakeet has been debated for the past 60 years (Fleming 1939, Nixon 1982, Triggs and Daugherty 1996). In spite of the marked differences that have been noted between Yellow-crowned and Forbes' Parakeets (morphology, vocalizations and allozyme electrophoresis data), the latter has been classified as a subspecies of Yellow-crowned Parakeet by most authorities (Forshaw 1989, Turbott 1990, Higgins 1999).

Our data resolve the relationship between Forbes' Parakeet and Yellow-crowned Parakeet with a high level of statistical support. The two criteria of diagnosability and monophyly required under the PSC are fulfilled, supporting the specific status of Forbes' Parakeet as *C. forbesi*. Based on our mitochondrial control region sequences, we classify Forbes' Parakeet as the most divergent New Zealand *Cyanoramphus* taxon (genetic distances of 6.85–7.87% to all other *Cyanoramphus* species). In our view, this warrants its elevation to full species status supporting Hypothesis 3 (see Introduction). For comparison, pairwise intergeneric comparisons for complete control region sequences from a wide variety of birds range from about 12 to 25% (Baker and Marshall 1997). Often quite small genetic distances are found between firmly established bird species (Grant and Grant 1997, Avise 1983). This observation suggests that relatively few genetic changes may be involved in avian speciation and that the phenotypic effects of these changes are minor (Snell 1991). Consistent with Snell (1991), morphological differences between Forbes' Parakeet and other *Cyanoramphus* species are rela-

tively small. Unlike the Orange-fronted Parakeet, the head colour of Forbes' Parakeet resembles that of the Yellow-crowned Parakeets, although it is more highly divergent. Thus, crown colour(s) may not be an entirely reliable taxonomic character.

Forbes' Parakeet is allopatric to its previously assigned conspecific Yellow-crowned Parakeet and therefore cannot be tested for reproductive isolation or compatibility of mate recognition systems according to the BSC and RSC; but using mitochondrial DNA data, we have fulfilled criteria for the PSC justifying specific status for Forbes' Parakeet. The Chatham Island subspecies of Red-crowned Parakeet *C. n. chathamensis* is the only sympatric congener of Forbes' Parakeet. One must therefore test the applicability of the BSC for Forbes' Parakeet based on its interactions with the Chatham Island Red-crowned Parakeet rather than the Yellow-crowned Parakeet. The BSC cannot be applied strictly in this case due to the high level of hybridization reported between Forbes' and Chatham Island Red-crowned Parakeet in the highly modified habitat on Mangere Island (Taylor 1975).

If the BSC were to be applied strictly and universally in avian systematics, many otherwise well-established species of parakeets and parrots would be judged to be conspecific. This is because post-zygotic isolating mechanisms appear to have evolved much later than pre-zygotic isolating mechanisms in the chronology of parrot speciation (Grant and Grant 1997). It is known that at least 7.5% (27 species) of *Psittaciformes* have bred with another species in the wild and produced fertile hybrids (Grant and Grant 1992). Thus, many parrot species, including Forbes' Parakeet and most other *Cyanoramphus* parakeets are actively speciating. Some have evolved effective pre-mating isolating mechanisms (e.g. Orange-fronted Parakeets), but may not have diverged far enough to satisfy the strict BSC definition. The RSC and CSC cannot be applied effectively in this case either. This is due to consideration of the extreme habitat modifications that have taken place on Mangere Island. Prior to deforestation of Mangere Island, Forbes' and the Chatham Island subspecies of Red-crowned Parakeets may not have been in contact, and may well have evolved effective ecological pre-mating isolating mechanisms based on differing habitat preferences. The absence of effective post-zygotic isolating mechanisms contributes to the present level of hybridization observed between these species. After deforestation, the modified vegetation (rank pasture) suited Chatham Island Red-crowned Parakeet better than Forbes' Parakeet and allowed rapid colonization of Mangere Island by the former. Forbes' Parakeet declined in numbers rapidly because of reduced opportunity for conspecific mate choice in the new habitat, ultimately leading to extensive hybridization with the Chatham Island Red-crowned Parakeet. The apparent lack of an effective pre-mating isolating mechanism in Forbes' Parakeet cannot therefore be used to test for species status. Nevertheless, we have presented evidence that a unique genotype is still present within Forbes' Parakeet, warranting recognition as a phylogenetic species. The conservation status of Forbes' Parakeet will not, however, be affected by its elevation to full species status because it is already being managed as a full species. Obtaining a larger sample size of DNA sequences would allow us to examine further the level of genetic cohesion that may exist within Forbes' Parakeet (Boon *et al.* unpubl.).

Phylogeny of Cyanoramphus

The phylogenetic trees (Figures 6–8) produced using independent phylogenetic inference methods are broadly congruent. This indicates that our overall phylogenetic inference is robust and that there is a high probability that our trees reflect the true biological history of the mitochondrial DNA control region. Minor differences in the branching order between the trees have been found. It is sometimes prudent to investigate variation at more than one gene when assessing taxonomic status (Norman *et al.* 1998). Our analyses using cytochrome *b* and control region provide different levels of phylogenetic resolution. They complement each other well and have no significant conflict between them. Although the loci are linked, conclusions supported by independent analysis of them increases the probability that we have inferred the correct phylogeny.

The phylograms (Figures 7 and 8) and cladogram (Figure 6) all show three well-supported major *Cyanoramphus* clades comprising Orange-fronted, Red-crowned and Yellow-crowned Parakeets. Ancestral to these are the Antipodes Island Parakeet and Forbes' Parakeet which form two relatively distant outgroups to the other species. Intraspecific variation within the main clades is highest for Red-crowned Parakeets, for which several well-described subspecies exist. The various Red-crowned Parakeet subspecies are sister species to the Orange-fronted Parakeet. Our data support the current subspecific classification of the Chatham Island Red-crowned subspecies, *C. n. chathamensis*. The captive CD1212 Red-crowned Parakeet appears to be genetically distinct from the other Red-crowned Parakeet taxa, but no further conclusions can be made without detailed information on its origin. The low level of genetic heterogeneity and lack of population genetic structure observed within what is presently described as Yellow-crowned Parakeet suggests a high level of panmixia and does not provide support for Triggs and Daugherty's (1996) hypothesis on the separation of Northern/Southern genotypes. Larger sample sizes and more extensive geographic sampling of Yellow-crowned Parakeets from the North Island of New Zealand might provide the level of phylogenetic resolution needed to support or refute this hypothesis more firmly. The Orange-fronted Parakeet clade is formed by individuals whose control region sequences are all almost identical to one another, showing extreme genetic cohesion, further supporting their status as members of a distinct species. Contrary to some classifications (Triggs and Daugherty 1996), the molecular data for Forbes' Parakeet show a remarkably high level of divergence from those of all other *Cyanoramphus* species. These values are higher than those for any other intrageneric comparisons and it may possibly be one of the most ancient *Cyanoramphus* taxa. However, genetic data from more Forbes' Parakeet individuals will need to be examined in order to obtain a final phylogenetic resolution with respect to its origins and to search for molecular signals left by hybridization with other species (Boon *et al.* unpubl.).

Conservation and summary

The Orange-fronted Parakeet is highly endangered. Its geographical distribution is extremely limited, and it is presently only known for certain from two beech *Nothofagus* forest valleys in North Canterbury (Kearvell 1997). One of these popu-

lations (Hope Valley) may now be extinct. The remaining known population in the Hurunui Valley may total less than 500 individuals (Kearvell 1997). Despite its uncertain taxonomic status and contrary to Taylor *et al.*'s (1986) conclusion, the Orange-fronted Parakeet is still maintained under Category "A" (highest priority for conservation action) by the New Zealand Department of Conservation (Tisdall 1994). This was done in order to ensure its conservation status would not be compromised before a taxonomic resolution was achieved. Our study confirms that this decision was prudent. Intensive management of its habitat by ongoing removal of stoats, deer, ferrets and possums is justified by the confirmation of its identity as a species under four different species concepts. Continued intensive management and ecological study of the small number of remaining birds is required for this species to recover from its endangered status.

Forbes' Parakeet is critically endangered. Numbers were once as low as 20–30 birds (Taylor 1975, 1985), and the current distribution is limited only to Mangere (112 ha) and Little Mangere (16 ha) Islands in the Chatham Group (Taylor 1975). The most recent population estimate indicates the number of Forbes' Parakeet to be approximately 100 (Mason *et al.* 1999). As numbers of Forbes' Parakeet declined, they hybridized with numerically superior Red-crowned Parakeets on Mangere Island, possibly due to breeding opportunities created by extensive modifications to vegetation from overgrazing, deforestation and introduction of predators (Taylor 1975). We have confirmed the species status of Forbes' Parakeet and, therefore, its population size should be closely monitored and active management of this species should be carried out to prevent its numbers declining further. Limiting numbers of Red-crowned and hybrid parakeets on Mangere Island, as is presently done, should continue to prevent genetic swamping of the Forbes' Parakeet gene pool. Our data show that, to date, such measures have effectively helped to preserve the genetic integrity of Forbes' Parakeet.

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References

- ABI Prism™ Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit Protocol (1997) by Perkin-Elmer Corporation, California.
- Anon (1997) *Lasergene biocomputing software for Windows version 4*. Madison, WI: DNASTAR.
- Awise, J. C. (1983) Commentary on George F. Barrowclough's "Biochemical studies of microevolutionary processes". Pp. 223–270 in A. H. Brush, and G. A. Clark Jr, eds. *Perspectives in ornithology*. New York: Cambridge University Press.

- Awise, J. C. and Nelson, W. S. (1988) Molecular genetic relationships of the extinct Dusky Seaside Sparrow. *Science* 243: 646–648.
- Baker, A. J. and Marshall, H. D. (1997) Mitochondrial control region sequences as tools for understanding evolution. Pp. 51–82 in D. P. Mindell, ed. *Avian molecular evolution and systematics*. San Diego: Academic Press.
- Baum, D. and Shaw, K. L. (1995) Genealogical perspectives on the species problem. *Monogr. Syst. Bot.* 53: 289–303.
- Birt, T. P., Friesen, V. L., Green, J. M., Montevecchi, W. A. and Davidson, W. S. (1992) Cytochrome *b* sequence variation among parrots. *Hereditas* 117: 67–72.
- Buller, W. L. R. (1869) On some new species of New Zealand birds. *Trans. N.Z. Inst.* 2: 390–392.
- Cabot, E. (1998) XESEE 3.2 Eyeball sequence editor software.
- Cottam, G. and Curtis, J. T. (1956) The use of distance measures in phytosociological sampling. *Ecology* 37: 451–460.
- Cracraft, J. (1983) Species concepts and speciation analysis. Pp. 159–187 in R. F. Johnston, ed. *Current Ornithology*, 1. New York: Plenum Press.
- Cracraft, J. (1997) Species concepts in systematics and conservation biology – an ornithological viewpoint. Pp. 325–339 in M. F. Claridge, H. A. Dawah and M. Wilson, eds. *Species: the units of biodiversity*. London: Chapman and Hall.
- Dawson, R. J. G. (1992) A molecular examination of some enigmatic birds. PhD thesis, University of Nottingham, U. K.
- Desjardins, P. and Morais, R. (1990) Sequence and gene organization of the chicken mitochondrial genome: a novel gene order in higher vertebrates. *Mol. Biol.* 212: 599–634.
- Edwards, S. V., Arctander, P. and Wilson, A. C. (1991) Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. *Proc. R. Soc. Lond. B.* 243: 99–107.
- Elliott, G. P., Dilks, P. J. and O'Donnell, C. F. J. (1996) The ecology of Yellow-crowned Parakeets (*Cyanoramphus auriceps*) in *Nothofagus* forest in Fiordland, New Zealand. *N. Z. J. Zool.* 23: 249–265.
- Falla, R. A., Sibson, R. B. and Turbott, E. G. (1966) *A field guide to the birds of New Zealand*, pp. 175–177. London: Collins.
- Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- Finsch, O. (1869) Remarks on some species of birds from New Zealand. *Trans. N.Z. Inst.* 2: 389–390.
- Fleming, C. A. (1939) Birds of the Chatham Islands, 2. *Emu* 38: 492–509.
- Forshaw, J. M. (1989) *Parrots of the world*, pp. 262–272. Melbourne, Australia: Landsdown.
- Grant, P. R. and Grant, B. R. (1992) Hybridisation of bird species. *Science* 256: 193–197.
- Grant, P. R. and Grant, B. R. (1997) Genetics and the origin of bird species. *Proc. Natl. Acad. Sci. U.S.A.* 94: 7768–7775.
- Gray, G. R. (1862) List of *Psittacidae* in the British Museum. *Ibis* 4: 229.
- Graybeal, A. (1995) Naming species. *Syst. Biol.* 44: 237–250.
- Greene, T. J. (1998) Foraging ecology of the Red-crowned Parakeet (*Cyanoramphus novaezelandiae novaezelandiae*) and Yellow-crowned Parakeets (*C. auriceps auriceps*) on Little Barrier Island, Hauraki Gulf, New Zealand. *N. Z. J. Zool.* 22: 161–172.
- Greig-Smith, P. (1964) *Quantitative plant ecology*. Second edition. Washington D.C.: Butterworth.
- Gyllensten, U., Wharton, D., Josefsson, A. and Wilson, A. C. (1991) Paternal inheritance of mitochondrial DNA in mice. *Nature* 352: 255–257.
- Harrison, M. (1970) The Orange-fronted Parakeet (*Cyanoramphus malherbi*). *Notornis* 17: 115–125.
- Helm-Bychowski, K. and Cracraft, J. (1993) Recovering phylogenetic signal from DNA sequences. Relationships within the Corvine assemblage (Class Aves) as inferred from

- complete sequences of the mitochondrial DNA cytochrome *b* gene. *Mol. Biol. Evol.* 10: 1196–1214.
- Higgins P. J., ed. (1999) *Handbook of Australian, New Zealand and Antarctic birds, 4: Parrots to Dollarbird*. Pp. 469–504. Melbourne, Australia: Oxford University Press.
- Holyoak, D. T. (1974) *Cyanoramphus malherbi*, is it a colour morph of *C. a. auriceps*? *Bull. Brit. Orn. Club* 94: 4–9.
- Irwin, D. M., Kocher, T. D. and Wilson, A. C. (1991) Evolution of the cytochrome *b* gene of mammals. *J. Mol. Evol.* 32: 128–144.
- Kearvell, J. (1997) *Cyanoramphus malherbi* and the colour morph question. Progress report for the 1995–1997 seasons. Unpubl. report, New Zealand Department of Conservation, Christchurch.
- Kearvell, J. (1999) Orange-fronted (*Cyanoramphus malherbi*) and Yellow-crowned (*C. auriceps*) Parakeets: Breeding during the winter of 1999 in North Canterbury, a preliminary investigation. Unpubl. report, New Zealand Department of Conservation, Christchurch.
- Kearvell, J. (2000) The use of transect counts and the Panel method for detecting trends in the population of the endangered Orange-fronted Parakeet (*Cyanoramphus malherbi*) in two North Canterbury valleys. Unpubl. report, New Zealand Department of Conservation, Christchurch.
- Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. and Wilson, A. C. (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6196–6200.
- Kumar, S., Tamura, K. and Nei, M. (1993) MEGA: molecular evolutionary genetics analysis, version 1.01. University Park, PA: Pennsylvania State University.
- Lansman, R. A., Avise, J. C. and Huettel, M. D. (1983) Critical experimental test of the possibility of “paternal leakage” of mitochondrial DNA. *Proc. Natl. Acad. Sci. U.S.A.* 80: 1969–1971.
- Leeton, P. R. J., Christidis, L., Westerman, M. and Boles, W. E. (1994) Molecular phylogenetic affinities of the night parrot (*Geopsittacus occidentalis*) and the ground parrot (*Pezoporus wallicus*). *Auk* 111: 833–843.
- Lopez, J. V., Yuhki, N., Masuda, R., Modi, W. and O’Brien, S. J. (1994) *Numt*, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. *J. Mol. Evol.* 39: 174–190.
- Mason, J., O’Connor, S., Baird, A., King, A. and King, S. (1999) Chatham Island update. *Rare Bits* 33: 13 (Unpublished quarterly update report, Biodiversity Recovery Unit, Department of Conservation, Wellington, New Zealand).
- Mayr, E. (1942) *Systematics and the origin of species*. Pp. 119–122. New York: Columbia University Press.
- Mayr, E. (1970) *Populations, species and evolution*. Pp. 10–20. Cambridge, MA: Harvard University Press.
- Mindell, D. P., Sorenson, M. D. and Dimcheff, D. E. (1998) Multiple independent origins of mitochondrial gene order in birds. *Proc. Natl. Acad. Sci. U.S.A.* 95: 10693–10697.
- Miyaki, C. Y., Mاتيoli, S. R., Burke, T. and Wajntal, A. (1998) Parrot evolution and paleogeographical events – mitochondrial DNA evidence. *Mol. Biol. Evol.* 15: 544–551.
- Moritz, C., Dowling, T. E. and Brown, W. M. (1987) Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annu. Rev. Ecol. Syst.* 18: 269–292.
- Nixon, A. J. (1981) The external morphology and taxonomic status of the Orange-fronted Parakeet. *Notornis* 28: 292–300.
- Nixon, A. J. (1982) Aspects of the ecology and morphology of *Cyanoramphus* parakeets

- and hybrids from Mangere Island, Chatham Islands. MSc thesis, Victoria University of Wellington, New Zealand.
- Norman, J. A., Christidis, L., Westerman, M. and Richard Hill, F. A. (1998) Molecular data confirms the species status of the Christmas Island Hawk-owl *Ninox natalis*. *Emu* 98: 197–208.
- Oliver, W. R. B. (1930) *New Zealand birds*, pp. 410–417. Wellington, New Zealand: Fine Arts New Zealand.
- Oliver, W. R. B. (1955) *New Zealand birds*, pp. 554–564. Wellington, New Zealand: A. H. & A. W. Reed.
- Paterson, H. E. H. (1985) The recognition concept of species. Pp. 21–29 in E. S. Vrba, ed. *Species and speciation*. Pretoria: Transvaal Museum (Monogr. 4).
- Pickard, J. (1990) Aspects of the behaviour and vocalisations of captive Antipodes Island parakeets (*Cyanoramphus unicolor*, Lear) and comparisons with other *Cyanoramphus* taxa. MSc thesis, Victoria University of Wellington, New Zealand.
- Quinn, T. W. (1997) Molecular evolution of the mitochondrial genome. Pp. 3–28 in D. P. Mindell, ed. *Avian molecular evolution and systematics*. San Diego: Academic Press.
- Rodríguez, J., Oliver, J. L., Marín, A. and Medina, J. R. (1990) The general stochastic model of nucleotide substitution. *J. Theor. Biol.* 142: 485–501.
- Rothschild, W. (1893) *Proc. Zool. Soc. London*: 529.
- Salvadori, T. (1891) *Catalogue of the birds of the British Museum* 20: 590.
- Sambrook, J.; Fritsch, E.F.; Maniatis, T. 1989. *Molecular cloning: a laboratory manual*. Second edition. New York: Cold Spring Harbor Laboratory Press.
- Simpson, G. G. (1961) *Principles of animal taxonomy*, pp. 152–155. New York: Columbia University Press.
- Smith, M. F., Thomas, W. K. and Patton, J. L. (1992) Mitochondrial DNA-like sequences in the nuclear genome of an akodontine rodent. *Mol. Biol. Evol.* 9: 204–215.
- Snell, R. R. (1991) Interspecific allozyme differentiation among North Atlantic white-headed larid gulls. *Auk* 108: 319–328.
- Sorenson, M. D. and Fleischer, R. C. (1996) Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. *Proc. Natl. Acad. Sci. U.S.A.* 93: 15239–15243.
- Souance, Ch. De. (1857) Scriptions de trois nouvelles espèces de Perroquets. *Rev. Mag. Zool.* IX: 98.
- Swofford, D. L. (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sunderland, MA: Sinauer Associates.
- Taylor, R. H. (1975) Some ideas on speciation in New Zealand parakeets. *Notornis* 22: 110–121.
- Taylor, R. H. (1985) Status, habits and conservation of *Cyanoramphus* parakeets in the New Zealand region. Pp. 195–211 in P. J. Moors, ed. *Conservation of island birds*. Cambridge, U. K.: International Council for Bird Conservation (Techn. Publ. 3).
- Taylor, R. H. (1998) A reappraisal of the Orange-fronted Parakeet (*Cyanoramphus* sp.) – species or colour morph? *Notornis* 45: 49–63.
- Taylor, R. H., Heatherbell, E. G. and Heatherbell, E. M. (1986) The Orange-fronted Parakeet (*Cyanoramphus malherbi*) is a colour morph of the Yellow-crowned Parakeet (*C. a. auriceps*). *Notornis* 33: 17–22.
- Templeton, A. R. (1989) The meaning of species and speciation: a genetic perspective. Pp. 3–27 in D. Otte and J. A. Endler, eds. *Speciation and its consequences*. Sunderland, MA: Sinauer Associates.
- Tisdall, C. (1994) *Setting priorities for the conservation of New Zealand's threatened plants and animals*, p. 26. New Zealand Department of Conservation, Wellington.
- Triggs, S. J. and Daugherty, C. H. (1996) Conservation and genetics of New Zealand parakeets. *Bird Conserv. Internatn* 6: 89–101.

Turbott, E. G. (1990) *Checklist of the birds of New Zealand and the Ross Dependency, Antarctica*. Third edition. Wellington, New Zealand: Random Century and Ornithological Society of New Zealand.

Zhang, D. X. and Hewitt, G. M. (1996) Nuclear integrations: challenges for mitochondrial DNA markers. *TREE* 11: 247-251.

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