Phylogenetic affinities of New Zealand white-chinned petrels: questions for conservation management

Kalinka Rexer-Huber & Bruce C. Robertson

Report for Objective 6D, Project POP2014-02

Department of Conservation, Conservation Services Programme

November 2015

Phylogenetic affinities of New Zealand white-chinned petrels: questions for conservation management

Department of Conservation, Conservation Services Programme project POP2014-02

Kalinka Rexer-Huber[1,2,*] and Bruce C. Robertson[1]

[1] Department of Zoology, University of Otago, PO Box 56, Dunedin New Zealand

[2] Parker Conservation, 126 Maryhill Tce, Dunedin New Zealand

[*] Corresponding author: kalinka.rexerhuber@gmail.com

Please cite as:

Rexer-Huber, K. and Robertson, B.C. 2015. Phylogenetic affinities of New Zealand white-chinned petrels: questions for conservation management. Report prepared by University of Otago for the New Zealand Department of Conservation, Wellington. 17p.

Contents

Abstract

White-chinned petrels are one of the most-affected seabird species in fisheries bycatch, but taxonomic uncertainty hinders risk assessment and species management efforts. The focus of the uncertainty is in the New Zealand region, so we tested whether any populations in the NZ region are distinct. We obtained DNA sequence data for the mitochondrial cytochrome *b* gene (~1143 base pairs, bp) and the nuclear intron β-fibrinogen 7th intron (~933 bp). Sequences were aligned and investigated using maximum-likelihood, Bayesian and distance analyses. These data did not support a separate taxon for the Antipodes breeding population. The apparent lack of island-level differences in the NZ region may indicate a NZ-regional genetic management unit, but should be assessed at a finer scale using nuclear microsatellite markers.

Introduction

White-chinned petrels were first described by Linnaeus as *Procellaria aequinoctialis* in 1758. Despite many taxonomic revisions, the current taxonomy has returned to *Procellaria aequinoctialis*, a single global taxon including birds from all breeding sites in the southern Atlantic, Indian and Pacific Oceans (Gill et al., 2010). However, two independent approaches have suggested that white-chinned petrels actually comprise two taxa (at sub-species rank), rather than one global taxon (Fraser, 2005; Techow, 2007).

Fraser (2005) conducted a morphometric study that suggested white-chinned petrels from the Antipodes Islands are sufficiently distinct from any of the other populations (among others, the Auckland Islands population) to be considered a subspecies. He proposed reinstating *P. a. steadi* Mathews for Antipodes white-chinned petrels, and that *P. a. aequinoctialis* Linnaeus be retained for the nominate subspecies. Building on that work, Techow (2007) used molecular genetics to assess phylogenetic relationships among white-chinned petrel populations. That approach also supported the idea of two white-chinned petrel taxa at the subspecies level, but the Antipodes placed together with Auckland Island birds forming a New Zealand (NZ) regional population. Subsequently, Techow and colleagues (2009) recommended using *P. a. steadi* Mathews for the New Zealand population.

Given the threat incidental bycatch mortalities pose to white-chinned petrels (e.g., Abraham & Thompson, 2014), reducing the uncertainty around white-chinned petrel taxonomy is a high priority (Wilson & Waugh, 2013). The key to the current taxonomic uncertainty is the disagreement in the geographic limits of the two white-chinned petrel taxa proposed by the morphometric and phylogenetic approaches. The ambiguity centres in the NZ region: is the Antipodes Island white-chinned petrel population distinct from the Auckland Island population? The geographic boundary is important to know because the number of observed captures around Antipodes and Auckland Islands differ (Abraham & Thompson, 2014), suggesting that some colonies may be impacted more than others. If there are genetic differences between white-chinned petrel populations in the NZ region, management scale should reflect the geographic boundaries of those populations.

So it is clearly important to reduce the uncertainty around white-chinned petrel taxonomy. Although it is difficult to reconcile the results from morphometric and genetic studies, key limitations in each study suggest opportunities to resolve the taxonomic uncertainty for white-chinned petrels.

Limitations and opportunities

Sample origins were a challenge faced by both morphometric and genetic studies of white-chinned petrel phylogeography (Fraser, 2005; Techow, 2007; also see Mischler et al., 2015). Phylogeographic studies require sampling from known geographic origins. However, known-origin material is often scarce, probably due to the logistical challenges involved in sampling nocturnal burrow-nesting seabirds that breed on remote subantarctic islands. Despite searching widely, Fraser (2005) found only 11 museum skins of white-chinned petrels labelled as coming from the Antipodes, six from Auckland Islands and none from Campbell Island. These were used as a known-origin sample set to assign 150 white-chinned petrels to breeding population. For example, bycaught birds that grouped in putative ‘Auckland’ and ‘Antipodes’ clusters were related to known-origin skins, then other bycaught birds were assigned to each cluster (Fraser, 2005). Similarly, only six samples of known origin from the Auckland Islands were available for Techow (2007), and none existed from Campbell Island. To

supplement the small number of known-origin individuals, samples from birds caught as bycatch from within 50 km of the Auckland Islands were included. The assumption that breeding birds caught near an island group must be from that island was again applied by Mischler et al. (2015) to cluster bycatch birds by proximity to islands.

Here we resolve uncertainty associated with sampling by using only samples from breeding birds or large chicks, by quadrupling the number of Auckland Island individuals included and by including the Campbell Island population for the first time. We also add a nuclear marker since mitochondrial DNA alone only represents phylogenetic patterns of the maternal lineage (Techow et al., 2009).

Aims and objectives

The primary aim of our work is to resolve the uncertainty in white-chinned petrel taxonomy by asking whether any populations in the New Zealand region are distinct. We look for island-specific genetic variation, as suggested by previous studies, testing the idea that Antipodes white-chinned petrels are distinct from Auckland Island birds.

Methods

Sampling

Tissue, feather or blood samples were collected from white-chinned petrel chicks and breeding adults at their breeding colonies at Auckland (n=30), Antipodes (n=23), Campbell (n=30), and Marion (n=20) Islands, Îles Crozet (n=21) and South Georgia (n=56) (appendix Table A1). Tissue and blood samples were stored in ethanol or lysis buffer. DNA extraction involved standard overnight Chelex-proteinase K digest of blood (5–20 µL) or tissue (~500 mg) followed by standard ethanol extraction (Barth et al., 2013).

Gene amplification and sequencing

We obtained DNA sequence data for the mitochondrial cytochrome *b* gene (1143 base pairs, bp) and the 7th intron of the nuclear β-fibrinogen gene (β-fibI7, 918 bp). We amplified a ~1200 bp fragment containing the entire cytochrome *b* (cyt *b*) gene using PCR primers H1-WCPfullcytb and L1-WCPfullcytb (5'-TTTTGGTTTACAAGACCAATGTTT-3' and 5'-TACAACTCATGGCAGCCAAA-3', respectively), designed from sequence amplified using primers L14675 and H16064 (Sorenson et al., 1999). A ~1000 bp fragment of the β-fibI7 gene was amplified with PCR primers FIB-BI7U and FIB-BI7L (Prychitko & Moore, 1997). For cyt *b*, amplifications were performed in a 25 µL reaction mix containing 25 ng DNA, 1x NH_4, 1.5mM $MgCl_2$, 0.2 mM dNTPs, 0.5 pmol of each primer, 0.1 µL BIOTAQ DNA polymerase (Bioline, London UK). Thermal cycling parameters were 3 min denaturation at 94°C, 10 cycles at 94°C for 20 sec with T_a°C for 25 sec (T_a°C = 60° declining by 1°C in each of the 10 cycles) and 70 sec at 72°C, followed by 25 cycles at 94°C for 20 sec, 25 sec at 50°C, and a final 70 sec at 72°C. The β-fibI7 fragments were amplified in a 20 µL reaction volume containing 20 ng DNA, 0.5 mM of each primer, 0.2 µL MyTaq Red DNA polymerase (Bioline) and 4 µL MyTaq reaction buffer. Thermal cycling conditions for β-fibI7 were 3 min at 94°C, 35 cycles at 94°C for 20 sec with annealing at 62°C for 25 sec, and a final extension at 72°C for 70 sec. Purified PCR products (AcroPrep 96 Filter Plates; Pall Corporation, Ann Arbor USA) were sequenced using forward and reverse primers for both genes using BigDye Terminator v.3.1 (ThermoFisher) on an ABI 3730xl DNA analyser (Applied Biosystems, Carlsbad USA).

Sequence analysis

The phylogenetic affinities of New Zealand's white-chinned petrels were investigated using mitochondrial and nuclear sequences: cytochrome *b* gene sequences (1143 bp, 99 samples) and β-fibrinogen 7th intron sequences (918 bp, 80 samples) (Table 1).

Table 1. Origin of white-chinned petrels included in this study. n refers to the number of complete sequences available from each location.

Location	Geographic region	Sample	n cyt *b*	n β-fib
Antipodes	New Zealand	blood	23	16
Auckland	New Zealand	blood	23	18
Campbell	New Zealand	blood	17	20
South Georgia	South Atlantic Ocean	blood	9	7
Marion	Southern Indian Ocean	tissue	12	10
Crozet	Southern Indian Ocean	blood	15	9

Sequences were aligned and edited in Geneious v6.1.8 (Kearse et al., 2012). Since nuclear copies of mitochondrial DNA or numts are an issue in avian blood mtDNA studies (Sorenson & Quinn, 1998), we translated mitochondrial sequences into amino acid profiles and checked for stop codons and ambiguous sites indicating heterozygosity. We also used previously published cyt *b* and β-fibI7 sequences (Nunn & Stanley, 1998; Pasko et al., 2011) to confirm sequence identity.

To check for biases in the data, we looked for substitution saturation via a maximum composite likelihood estimate of transition/transversion following Tamura et al. (2004), and checked base composition consistency among taxa by computing the pattern disparity index following Kumar and Gadagkar (2001). These analyses were conducted in MEGA6 (Tamura et al., 2013).

Phylogenetic analysis

Phylogenetic analysis was performed using haplotypes from 30 sequences (1142 positions, cyt *b*) and 27 sequences (878 positions, β-fibI7), constructing trees using more than one method since algorithms and assumptions differ. In comparing trees for robustness and congruence, we assume that recurring relationships are true. Trees were constructed from alignments using maximum-likelihood (ML), and Bayesian analysis (BA) methods, using sequence from procellariid seabirds to root the trees (*Phoebastria nigripes* GenBank accession No. EF552760 and *Puffinus tenuirostris* AY695220 for β-fibI7; *Procellaria westlandica* AF076078 and *Procellaria parkinsoni*, AF076077 for cyt *b*) (Fain & Houde, 2004; Nunn & Stanley, 1998; Pasko et al., 2011). The best fit model of nucleotide evolution was HKY+I (cyt *b*) and HKY+Γ+I (β-fibI7) using AIC values in jModelTest 2 (Darriba et al., 2012).

Construction of ML trees began with a stepwise-addition starting tree, with bootstrap resampling (100 replicates) to assess support for internal branches in PAUP* 4.0b10 (Swofford, 2003). The distribution of rates at variable sites was equal and gamma-distributed for cyt *b* and β-fibI7, respectively. ML estimates of the shape parameter for gamma (Γ) rate variation among sites was 0.77 for β-fibI7. The genetic distance among taxa was determined in MEGA6 using the Maximum Composite Likelihood model (Tamura et al., 2004), with complete deletion of uncertain positions and including a gamma shape parameter of 0.77 for β-fibI7 following recommendations in Fregin et al. (2012). BA trees were constructed with 1.1 million generations per run and four parallel Monte Carlo

Markov (MCMC) chains, computed with a burn-in of 275,000 trees in MrBayes 3.2.1 (Huelsenbeck & Ronquist, 2001), including four gamma categories (+Γ) for β-fibI7.

Results

Sequence variation

The complete cytochrome *b* alignment consisted of 30 nucleotide sequences (28 white-chinned petrel and two GenBank sequences), with a total of 1142 positions in the final dataset. The sequences could be fully translated using the vertebrate mitochondrial code and did not contain non-sense or stop codons. Within the 28 white-chinned petrel sequences, there were 25 (2.2%) nucleotide positions where a base substitution occurred, with 12 (1.0%) parsimony-informative (PI) sites. Average base composition was biased, with a deficiency of guanine [28.33% (A), 27.07% (T/U), 32.03% (C), and 12.57% (G)] as expected for mitochondrial DNA. There was no saturation based on plots and no significant disparity in nucleotide composition, confirming homogenous sequence evolution.

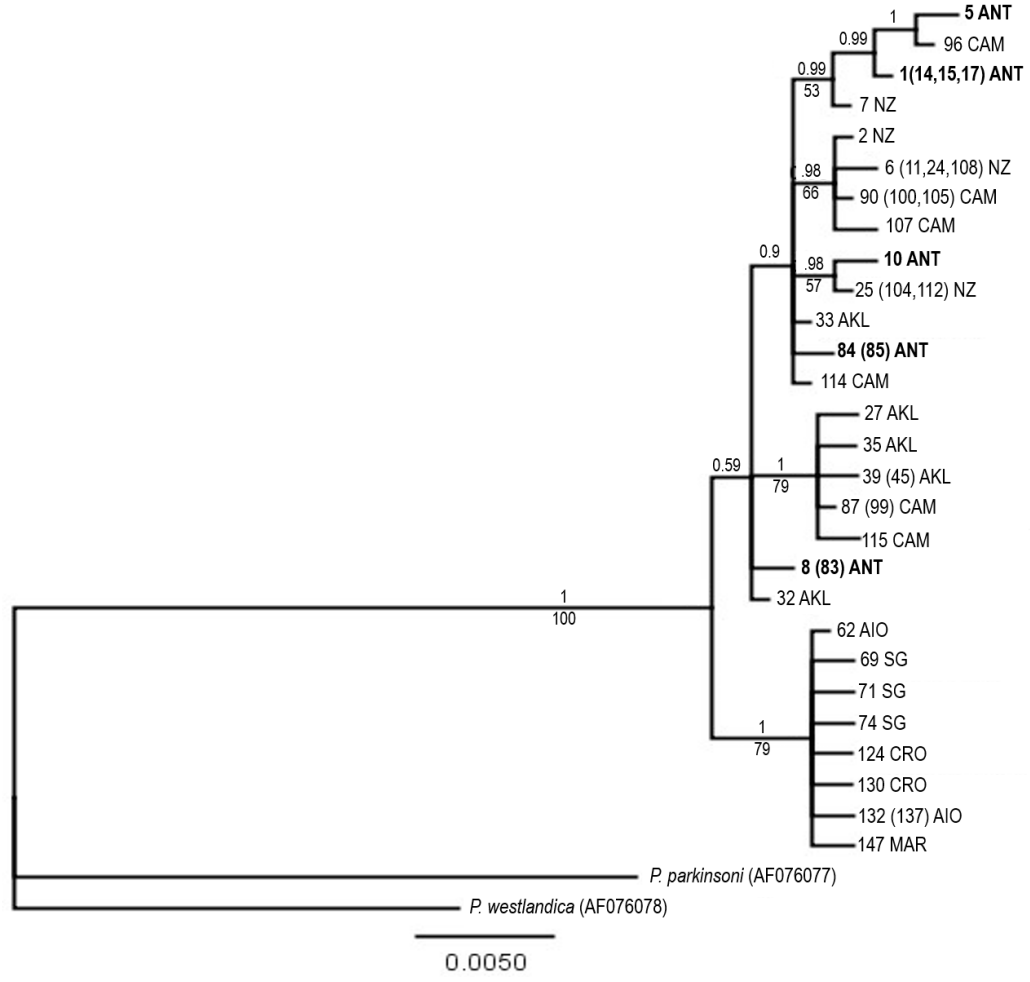

Figure 1. White-chinned petrel cytochrome *b* Bayesian inference tree, with posterior probability above branches and maximum-likelihood bootstrap consensus supports below branches (model HKY85+I). White-chinned petrel haplotypes named according to sampling location as follows: Antipodes (ANT), Auckland (AKL), Campbell (CAM), South Georgia (SG), Marion (MAR), Crozet (CRO), New Zealand sector (NZ), Atlantic/Indian Ocean sector (AIO). Numbers in brackets refer to sample information (Table A1).

There were no shared haplotypes between white-chinned petrels from the NZ region (Antipodes, Auckland and Campbell Island) and Atlantic-Indian Ocean (AIO) colonies (South Georgia, Marion and Crozet) (Fig. 1). Genetic distances between NZ regional and Atlantic-Indian Ocean (AIO) clades were 0.006-0.007 (Table 2). The NZ region could not be separated further: haplotypes of Antipodes birds were found throughout the NZ regional clade (**bold** in Fig. 1) and did not fall out into any separate grouping relative to other NZ island populations. Genetic distances between haplotypes of birds from New Zealand islands were variable, ranging from <0.001 to 0.007, and the New Zealand islands had more haplotypes (20) than did the AIO islands (8 haplotypes) (Fig. 1).

The β-fibrinogen intron 7 alignment consisted of 27 nucleotide sequences (25 white-chinned petrel and two GenBank sequences), with a total of 878 positions in the final dataset. Within the 25 white-chinned petrel sequences, 18 nucleotide changes were identified with 14 PI sites. The nucleotide frequencies were 32.75% (A), 30.11% (T/U), 19.70% (C), and 17.45% (G). There was no significant disparity in β-fibI7 nucleotide composition. Haplotypes of Antipodes white-chinned petrels were found throughout the β-fibI7 tree (**bold** in Fig. 2), and did not separate from other populations (Fig. 2). The split between NZ and AIO regions was not observed in the β-fibI7 intron, with four haplotypes shared across all oceanic basins (Fig. 2, Table 3).

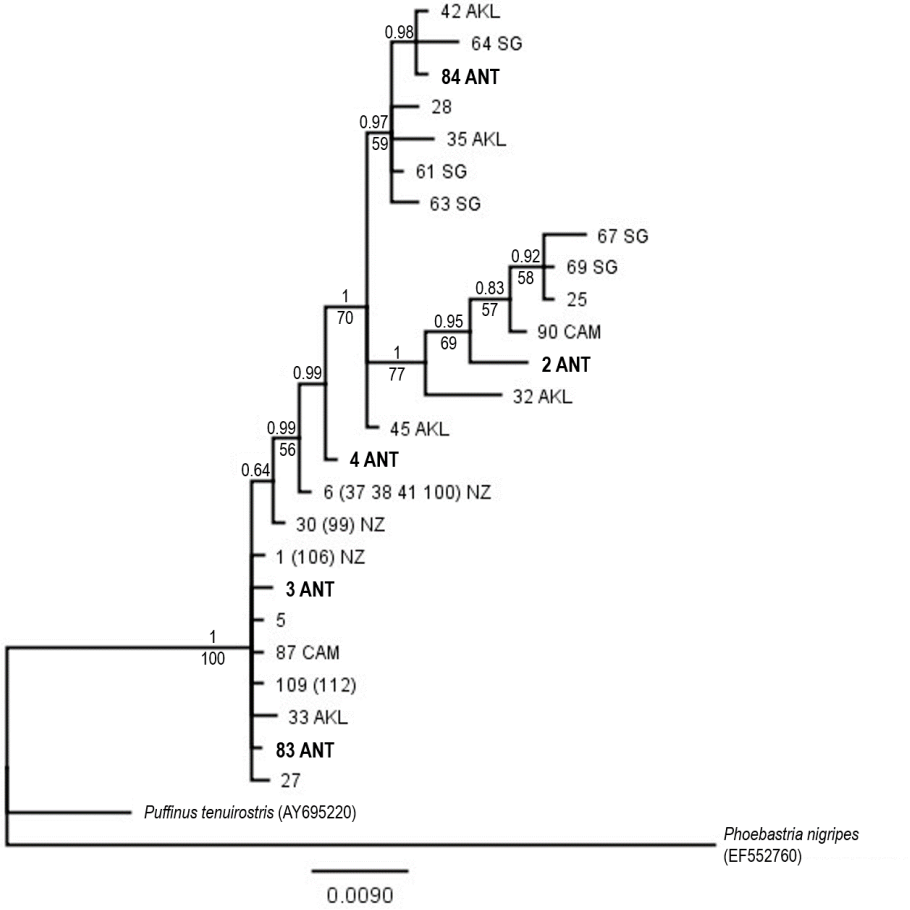

Figure 2. White-chinned petrel β-fibrinogen intron 7. Bayesian inference tree with posterior probability supports above branches and maximum-likelihood bootstrap consensus supports below the line (model HKY85+I+Γ, shape parameter 0.77). White-chinned petrel haplotypes named according to sampling location as follows: Antipodes (ANT), Auckland (AKL), Campbell (CAM), New Zealand regional (NZ), South Georgia (SG). Haplotypes 5, 25, 27 and 28 were found in birds from a combination of NZ and SG, Marion and Crozet Islands. Numbers in brackets refer to sample information (Table A1).

Table 2. Estimates of evolutionary divergence between white-chinned petrel cytochrome *b* sequences, based on compositional maximum likelihood distances of the entire cytochrome *b* gene. The number of base substitutions per site between sequences is shown. Numbers in brackets refer to samples in Table A1 or sequence accession numbers.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	Parkinsoni (AF076077)																													
2	Westlandica (AF076078)	0.036																												
3	1 ANT (14,15,17)	0.048	0.042																											
4	2 NZ	0.049	0.041	0.003																										
5	5_ANT	0.050	0.044	0.002	0.004																									
6	6 NZ (11,24,108)	0.050	0.042	0.004	0.001	0.005																								
7	7 NZ	0.049	0.041	0.001	0.002	0.003	0.003																							
8	8 ANT (83)	0.048	0.040	0.004	0.003	0.005	0.004	0.003																						
9	10 ANT	0.048	0.040	0.004	0.003	0.005	0.004	0.003	0.004																					
10	25 NZ (104,112)	0.049	0.041	0.003	0.002	0.004	0.003	0.002	0.003	0.001																				
11	27 AKL	0.050	0.042	0.005	0.004	0.007	0.005	0.004	0.004	0.005	0.004																			
12	32 AKL	0.047	0.039	0.003	0.002	0.004	0.003	0.002	0.001	0.003	0.002	0.003																		
13	33 AKL	0.048	0.040	0.002	0.001	0.004	0.002	0.001	0.002	0.002	0.001	0.004	0.001																	
14	35 AKL	0.048	0.040	0.005	0.004	0.007	0.005	0.004	0.004	0.005	0.004	0.002	0.003	0.004																
15	39 AKL (45)	0.049	0.042	0.005	0.004	0.007	0.005	0.004	0.004	0.005	0.004	0.002	0.003	0.004	0.002															
16	62 AIO	0.049	0.041	0.006	0.005	0.006	0.006	0.005	0.004	0.006	0.005	0.006	0.004	0.004	0.006	0.006														
17	69 SG	0.048	0.040	0.007	0.006	0.007	0.007	0.006	0.005	0.007	0.006	0.007	0.004	0.005	0.007	0.007	0.001													
18	71 SG	0.050	0.042	0.007	0.006	0.007	0.007	0.006	0.005	0.007	0.006	0.007	0.004	0.005	0.007	0.007	0.001	0.002												
19	74 SG	0.050	0.042	0.007	0.006	0.007	0.007	0.006	0.005	0.007	0.006	0.007	0.004	0.005	0.007	0.007	0.001	0.002	0.002											
20	84 ANT (85)	0.047	0.039	0.003	0.002	0.004	0.003	0.002	0.003	0.003	0.002	0.004	0.002	0.001	0.003	0.004	0.005	0.006	0.006	0.006										
21	87 CAM (99)	0.049	0.041	0.004	0.004	0.006	0.004	0.004	0.003	0.004	0.004	0.001	0.002	0.003	0.001	0.001	0.005	0.006	0.006	0.006	0.004									
22	90 CAM (100,105)	0.049	0.041	0.003	0.000	0.004	0.001	0.002	0.003	0.003	0.002	0.004	0.002	0.001	0.004	0.004	0.005	0.006	0.006	0.006	0.002	0.004								
23	96 CAM	0.049	0.043	0.001	0.004	0.001	0.004	0.002	0.004	0.004	0.004	0.006	0.004	0.003	0.006	0.006	0.005	0.006	0.006	0.006	0.004	0.005	0.004							
24	107 CAM	0.048	0.042	0.004	0.001	0.005	0.002	0.003	0.004	0.004	0.003	0.005	0.003	0.002	0.005	0.005	0.006	0.007	0.007	0.007	0.003	0.004	0.001	0.004						
25	114 CAM	0.048	0.040	0.002	0.001	0.004	0.002	0.001	0.002	0.002	0.001	0.004	0.001	0.000	0.004	0.004	0.004	0.005	0.005	0.005	0.001	0.003	0.001	0.003	0.002					
26	115 CAM	0.050	0.042	0.005	0.004	0.007	0.005	0.004	0.004	0.005	0.004	0.002	0.003	0.004	0.002	0.002	0.006	0.007	0.007	0.007	0.004	0.001	0.004	0.006	0.005	0.004				
27	124 CRO	0.048	0.042	0.005	0.006	0.005	0.007	0.006	0.005	0.007	0.006	0.007	0.004	0.005	0.007	0.007	0.001	0.002	0.002	0.002	0.006	0.006	0.006	0.004	0.007	0.005	0.007			
28	130 CRO	0.049	0.041	0.007	0.006	0.007	0.007	0.006	0.005	0.006	0.006	0.007	0.004	0.005	0.007	0.007	0.001	0.002	0.002	0.002	0.006	0.006	0.006	0.006	0.007	0.005	0.007	0.002		
29	132 AIO (137)	0.049	0.041	0.007	0.006	0.007	0.007	0.006	0.005	0.007	0.006	0.007	0.004	0.005	0.007	0.007	0.001	0.002	0.002	0.002	0.006	0.006	0.006	0.006	0.007	0.005	0.007	0.002	0.002	
30	147 MAR	0.050	0.042	0.007	0.006	0.007	0.007	0.006	0.005	0.007	0.006	0.007	0.004	0.005	0.007	0.007	0.001	0.002	0.002	0.002	0.006	0.006	0.006	0.006	0.007	0.005	0.007	0.002	0.002	0.002

Haplotypes named by sampling location: Antipodes (ANT), Auckland (AKL), Campbell (CAM), South Georgia (SG), Marion (MAR), Crozet (CRO), New Zealand sector (NZ), Atlantic/Indian Ocean sector (AIO)

Table 3. Estimates of evolutionary divergence between white-chinned petrel β-fibrinogen intron 7 sequences, based on compositional maximum likelihood distances of the entire 7th intron of β-fibrinogen. The number of base substitutions per site between sequences is shown. Numbers in brackets refer to samples in Table A1 or sequence accession numbers.

		1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***23***	***24***	***25***	***26***
1	*Phoebastria nigripes* (EF552760)																										
2	*Puffinus tenuirostris* (AY695220)	0.052																									
3	1 (106) NZ	0.057	0.023																								
4	2 ANT	0.070	0.034	0.010																							
5	3 ANT	0.059	0.024	0.001	0.010																						
6	4 ANT	0.060	0.026	0.003	0.010	0.002																					
7	5	0.057	0.023	0.000	0.010	0.001	0.003																				
8	6 (37 38 41 100) NZ	0.060	0.025	0.002	0.009	0.001	0.001	0.002																			
9	25	0.057	0.023	0.000	0.010	0.001	0.003	0.000	0.002																		
10	27	0.057	0.023	0.000	0.010	0.001	0.003	0.000	0.002	0.000																	
11	28	0.066	0.031	0.008	0.010	0.007	0.005	0.008	0.006	0.008	0.008																
12	30 (99) NZ	0.059	0.024	0.001	0.010	0.000	0.002	0.001	0.001	0.001	0.001	0.007															
13	32 AKL	0.067	0.033	0.009	0.008	0.009	0.009	0.009	0.010	0.009	0.009	0.009	0.009														
14	33 AKL	0.057	0.023	0.000	0.010	0.001	0.003	0.000	0.002	0.000	0.000	0.008	0.001	0.009													
15	35 AKL	0.064	0.030	0.007	0.009	0.006	0.006	0.007	0.007	0.007	0.007	0.003	0.006	0.008	0.007												
16	42 AKL	0.064	0.030	0.007	0.009	0.006	0.003	0.007	0.005	0.007	0.007	0.001	0.006	0.008	0.007	0.002											
17	45 AKL	0.063	0.029	0.006	0.008	0.005	0.002	0.006	0.003	0.006	0.006	0.002	0.005	0.007	0.006	0.003	0.001										
18	61 SG	0.064	0.030	0.007	0.009	0.006	0.003	0.007	0.005	0.007	0.007	0.001	0.006	0.008	0.007	0.002	0.000	0.001									
19	63 SG	0.066	0.031	0.008	0.009	0.007	0.005	0.008	0.006	0.008	0.008	0.002	0.007	0.009	0.008	0.003	0.001	0.002	0.001								
20	64 SG	0.064	0.030	0.007	0.009	0.006	0.003	0.007	0.005	0.007	0.007	0.001	0.006	0.008	0.007	0.002	0.000	0.001	0.000	0.001							
21	67 SG	0.068	0.034	0.010	0.005	0.010	0.009	0.010	0.010	0.010	0.010	0.009	0.010	0.006	0.010	0.008	0.008	0.007	0.008	0.008	0.008						
22	69 SG	0.068	0.034	0.010	0.005	0.010	0.009	0.010	0.010	0.010	0.010	0.009	0.010	0.006	0.010	0.008	0.008	0.007	0.008	0.008	0.008	0.000					
23	83 ANT	0.057	0.023	0.000	0.010	0.001	0.003	0.000	0.002	0.000	0.000	0.008	0.001	0.009	0.000	0.007	0.007	0.006	0.007	0.008	0.007	0.010	0.010				
24	84 ANT	0.064	0.030	0.007	0.009	0.006	0.003	0.007	0.005	0.007	0.007	0.001	0.006	0.008	0.007	0.002	0.000	0.001	0.000	0.001	0.000	0.008	0.008	0.007			
25	87 CAM	0.068	0.034	0.010	0.005	0.010	0.009	0.010	0.010	0.010	0.010	0.009	0.010	0.006	0.010	0.008	0.008	0.007	0.008	0.008	0.008	0.000	0.000	0.010	0.008		
26	90 CAM	0.068	0.033	0.009	0.002	0.009	0.009	0.009	0.008	0.009	0.009	0.009	0.009	0.007	0.009	0.009	0.008	0.007	0.008	0.008	0.008	0.002	0.002	0.009	0.008	0.002	
27	109 (112)	0.059	0.024	0.001	0.010	0.000	0.002	0.001	0.001	0.001	0.001	0.007	0.000	0.009	0.001	0.006	0.006	0.005	0.006	0.007	0.006	0.010	0.010	0.001	0.006	0.010	0.009

Haplotypes named by sampling location: Antipodes (ANT), Auckland (AKL), Campbell (CAM), New Zealand regional (NZ), South Georgia (SG). Haplotypes 5, 25, 27 and 28 were found in birds from a combination of NZ and SG, Marion and Crozet Islands.

Discussion

White-chinned petrels from the Antipodes and Auckland Islands group together, supporting the idea of a New Zealand regional taxon genetically distinct to the remainder of the global population (Techow et al., 2009). The inclusion of Campbell Island birds corroborates the pattern of a NZ regional taxon noted using Antipodes and Auckland Island birds.

We see no genetic support for morphometric differentiation of Antipodes and Auckland Island white-chinned petrels documented in bycatch birds by Fraser (2005) and Mischler et al. (2015). This does not mean the morphometric conclusions are incorrect, since phenotypic differences can exist without corresponding genetic variance (e.g., Mason & Taylor, 2015; Questiau et al., 1998). It would, however, be valuable to revisit morphometric studies using measurements from white-chinned petrels on breeding islands. This would rule out possible assignment biases, since it is not known whether museum skins used by Fraser (2005) were birds originally caught on the ground on specific islands.

The small genetic distances in the cytochrome *b* gene between Antipodes and Auckland groups (cyt *b*, 0.001-0.005) suggest little differentiation between birds on these islands. By contrast, we see distances of 0.006-0.007 (cyt *b*) between NZ white-chinned petrels and Atlantic and Indian Ocean white-chinned petrels (South Georgia, Marion and Crozet Islands). Although there is some discussion around the use of genetic distances (e.g., Fregin et al., 2012), we follow best-practise recommendations and use distances conservatively. Genetic distances are only used here to suggest management implications and identify gaps for further work, not used to underpin taxonomic revision.

The apparent lack of island-level differences may be the result of substantial gene flow within the NZ region, similar to the case of grey-faced petrels *Pterodroma macroptera* (Lawrence et al., 2014). Although there is a suggestion of a finer clade within the NZ region in cyt *b*, it has little support and is not useful from a management perspective since it cannot be distinguished geographically. Our results suggest one management unit in the NZ region. To test this, the next step is to use fine-resolution molecular markers (mtDNA control region and nuclear microsatellites) to tease out any island-level differences within the NZ region that could affect the definition of management units.

Although we found no genetically distinct white-chinned petrel populations in the NZ region, we suggest that tracking data should be considered when assessing the impacts of fisheries bycatch. The suggestion that Antipodes and Auckland birds are caught in different areas (Mischler et al., 2015) appears to be supported by tracking data; preliminary analyses show different foraging hotspots for breeding Antipodes and Auckland Island white-chinned petrels (D. Thompson & KRH unpublished data).

In conclusion, Antipodes Island white-chinned petrels are not genetically distinct from Auckland and Campbell Island birds, hence the Antipodes population does not warrant a subspecific identification (cf. Fraser, 2005). These findings support the idea that white-chinned petrels in the NZ region comprise their own taxon, genetically distinct from the remaining global white-chinned petrels. Moving forward, we suggest that the variability within the NZ regional population is sufficient to warrant using nuclear microsatellite markers to look more closely at island-level differences.

Acknowledgements

This work was supported by funding from NZ Department of Conservation's Conservation Services Programme project POP2014-02 and an Otago Museum Linnaeus Taxonomy Fellowship. The University of Otago provided funding and facilities, and NIWA contributed logistical support. Samples were generously provided by David Thompson, Kath Walker and Graeme Elliott (Antipodes), Peter Ryan (Marion); Richard Phillips (South Georgia) and Yves Cherel (Crozet). Campbell and Auckland Island samples could not have been collected without the support of Henk Haazen of SV *Tiama*. This work was conducted under permits No. 38414-FAU, 38027-LND and 40203-LND from the NZ Department of Conservation and approval No. 60/14 from the University of Otago Animal Ethics Committee.

References

Abraham, E. R., & Thompson, F. N. (2014). Captures of white-chinned petrel in trawl fisheries, in the New Zealand Exclusive Economic Zone, from 2002-03 to 2012-13. From https://data.dragonfly.co.nz/psc/v20140201/white-chinned-petrel/trawl/all-vessels/eez/all/, version dated 7 August 2015

Barth, J. M., Matschiner, M., & Robertson, B. C. (2013). Phylogenetic position and subspecies divergence of the endangered New Zealand dotterel (*Charadrius obscurus*). *PLoS ONE, 8*, e78068.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods, 9*, 772.

Fain, M. G., & Houde, P. (2004). Parallel radiations in the primary clades of birds. *Evolution, 58*, 2558-2573.

Fraser, M. J. (2005). *Characteristics of White-chinned petrels* Procellaria aequinoctialis *Linnaeus in New Zealand waters.* MSc, Massey University, Palmerston North.

Fregin, S., Haase, M., Olsson, U., & Alstrom, P. (2012). Pitfalls in comparisons of genetic distances: A case study of the avian family Acrocephalidae. *Molecular Phylogenetics and Evolution, 62*, 319-328.

Gill, B. J., Bell, B. D., Chambers, G. K., Medway, D. G., Palma, R. L., Scofield, R. P., Tennyson, A. J. D., & Worthy, T. H. (2010). *Checklist of the Birds of New Zealand, Norfolk and Macquarie Islands, and the Ross Dependency, Antarctica* (4th ed.). Wellington: Te Papa Press and OSNZ.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics, 17*, 754-755.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28*, 1647-1649.

Kumar, S., & Gadagkar, S. R. (2001). Disparity Index: A simple statistic to measure and test the homogeneity of substitution patterns between molecular sequences. *Genetics, 158*, 1321-1327.

Lawrence, H. A., Lyver, P. O., & Gleeson, D. M. (2014). Genetic panmixia in New Zealand's Grey-faced Petrel: implications for conservation and restoration. *Emu, 114*, 249-258.

Mason, N. A., & Taylor, S. A. (2015). Differentially expressed genes match bill morphology and plumage despite largely undifferentiated genomes in a Holarctic songbird. *Molecular Ecology, 24*, 3009-3025.

Mischler, C. P., Robertson, C. J. R., & Bell, E. A. (2015). Gender and geographic variation in morphometrics of white-chinned petrels (*Procellaria aequinoctialis*) in New Zealand and their foraging activities as determined from fisheries bycatch. *Notornis, 62*, 63-70.

Nunn, G. B., & Stanley, S. E. (1998). Body size effects and rates of cytochrome b evolution in tube-nosed seabirds. *Molecular Biology and Evolution, 15*, 1360-1371.

Pasko, L., Ericson, P. G., & Elzanowski, A. (2011). Phylogenetic utility and evolution of indels: A study in neognathous birds. *Molecular Phylogenetics and Evolution, 61*, 760-771.

Prychitko, T. M., & Moore, W. S. (1997). The utility of DNA sequences of an intron from the β-fibrinogen gene in phylogenetic analysis of woodpeckers (Aves: Picidae). *Molecular Phylogenetics and Evolution, 8*, 193-204.

Questiau, S., Eybert, M. C., Gaginskaya, A. R., Gielly, L., & Taberlet, P. (1998). Recent divergence between two morphologically differentiated subspecies of bluethroat (Aves: Muscicapidae: *Luscinia svecica*) inferred from mitochondrial DNA sequence variation. *Molecular Ecology, 7*, 239-245.

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T., & Mindell, D. P. (1999). Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution, 12*, 105-114.

Sorenson, M. D., & Quinn, T. W. (1998). Numts: a challenge for avian systematics and population biology. *The Auk, 115*, 214-221.

Swofford, D. L. (2003). *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sunderland USA: Sinauer Associates.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA), 101*, 11030-11035.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution, 30*, 2725-2729.

Techow, N. M. S. M. (2007). *Phylogeny and phylogeography of four Southern Ocean petrels.* PhD, University of Cape Town, Cape Town.

Techow, N. M. S. M., Ryan, P. G., & O'Ryan, C. (2009). Phylogeography and taxonomy of White-chinned and Spectacled Petrels. *Molecular Phylogenetics and Evolution, 52*(1), 25-33.

Appendix

Table A1. Details of all white-chinned petrel samples included in this study

ID No.	Sampling location	Geographic region	Sample type	Source
wcp001	Antipodes	New Zealand	blood	DT
wcp002	Antipodes	New Zealand	blood	DT
wcp003	Antipodes	New Zealand	blood	DT
wcp004	Antipodes	New Zealand	blood	DT
wcp005	Antipodes	New Zealand	blood	DT
wcp006	Antipodes	New Zealand	blood	DT
wcp007	Antipodes	New Zealand	blood	DT
wcp008	Antipodes	New Zealand	blood	DT
wcp009	Antipodes	New Zealand	blood	DT
wcp010	Antipodes	New Zealand	blood	DT
wcp011	Antipodes	New Zealand	blood	DT
wcp012	Antipodes	New Zealand	blood	DT
wcp013	Antipodes	New Zealand	blood	DT
wcp014	Antipodes	New Zealand	blood	DT
wcp015	Antipodes	New Zealand	blood	DT
wcp016	Antipodes	New Zealand	blood	DT
wcp017	Antipodes	New Zealand	blood	DT
wcp018	Antipodes	New Zealand	blood	DT
wcp019	Antipodes	New Zealand	blood	DT
wcp020	Antipodes	New Zealand	blood	DT
wcp083	Antipodes	New Zealand	blood	KW
wcp084	Antipodes	New Zealand	blood	KW
wcp085	Antipodes	New Zealand	blood	KW
wcp022	Auckland (Adams Isl)	New Zealand	blood	this study
wcp023	Auckland (Adams Isl)	New Zealand	blood	this study
wcp024	Auckland (Adams Isl)	New Zealand	blood	this study
wcp025	Auckland (Adams Isl)	New Zealand	blood	this study
wcp026	Auckland (Adams Isl)	New Zealand	blood	this study
wcp027	Auckland (Adams Isl)	New Zealand	blood	this study
wcp028	Auckland (Adams Isl)	New Zealand	blood	this study
wcp030	Auckland (Adams Isl)	New Zealand	blood	this study
wcp031	Auckland (Adams Isl)	New Zealand	blood	this study
wcp032	Auckland (Adams Isl)	New Zealand	blood	this study
wcp033	Auckland (Adams Isl)	New Zealand	blood	this study
wcp035	Auckland (Adams Isl)	New Zealand	blood	this study
wcp036	Auckland (Adams Isl)	New Zealand	blood	this study
wcp037	Auckland (Adams Isl)	New Zealand	blood	this study
wcp038	Auckland (Adams Isl)	New Zealand	blood	this study
wcp039	Auckland (Adams Isl)	New Zealand	blood	this study

ID No.	Sampling location	Geographic region	Sample type	Source
wcp040	Auckland (Adams Isl)	New Zealand	blood	this study
wcp041	Auckland (Adams Isl)	New Zealand	blood	this study
wcp042	Auckland (Adams Isl)	New Zealand	blood	this study
wcp043	Auckland (Adams Isl)	New Zealand	blood	this study
wcp045	Auckland (Adams Isl)	New Zealand	blood	this study
wcp047	Auckland (Adams Isl)	New Zealand	blood	this study
wcp049	Auckland (Adams Isl)	New Zealand	blood	this study
wcp087	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp090	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp093	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp096	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp097	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp098	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp099	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp100	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp101	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp102	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp103	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp104	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp105	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp106	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp107	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp108	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp109	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp111	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp112	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp114	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp115	Campbell (Monowai, Dent Isl)	New Zealand	blood	this study
wcp061	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp062	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp063	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp064	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp065	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp066	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp067	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp068	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp069	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp071	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp072	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp073	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp074	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp075	South Georgia (Bird Isl)	South Atlantic	blood	RP
wcp076	Marion	Southern Indian ocean	tissue	PR
wcp080	Marion	Southern Indian ocean	tissue	PR
wcp137	Marion	Southern Indian ocean	tissue	PR

ID No.	Sampling location	Geographic region	Sample type	Source
wcp138	Marion	Southern Indian ocean	tissue	PR
wcp139	Marion	Southern Indian ocean	tissue	PR
wcp140	Marion	Southern Indian ocean	tissue	PR
wcp141	Marion	Southern Indian ocean	tissue	PR
wcp143	Marion	Southern Indian ocean	tissue	PR
wcp144	Marion	Southern Indian ocean	tissue	PR
wcp145	Marion	Southern Indian ocean	tissue	PR
wcp147	Marion	Southern Indian ocean	tissue	PR
wcp149	Marion	Southern Indian ocean	tissue	PR
wcp116	Crozet	Southern Indian ocean	blood	YC
wcp117	Crozet	Southern Indian ocean	blood	YC
wcp118	Crozet	Southern Indian ocean	blood	YC
wcp119	Crozet	Southern Indian ocean	blood	YC
wcp120	Crozet	Southern Indian ocean	blood	YC
wcp121	Crozet	Southern Indian ocean	blood	YC
wcp122	Crozet	Southern Indian ocean	blood	YC
wcp123	Crozet	Southern Indian ocean	blood	YC
wcp124	Crozet	Southern Indian ocean	blood	YC
wcp126	Crozet	Southern Indian ocean	blood	YC
wcp127	Crozet	Southern Indian ocean	blood	YC
wcp128	Crozet	Southern Indian ocean	blood	YC
wcp130	Crozet	Southern Indian ocean	blood	YC
wcp131	Crozet	Southern Indian ocean	blood	YC
wcp132	Crozet	Southern Indian ocean	blood	YC

Source: DT David Thompson, NIWA; RP Richard Phillips, British Antarctic Survey; PR Peter Ryan, University of Cape Town; YC Yves Cherel, Centre National de la Recherche Scientifique France