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Phylogeographic structure and a genetic assignment method for Buller's albatross ssp. (*Thalassarche bulleri* ssp.)

JANA R. WOLD*
School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington 6140, New Zealand
Current Address: School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8041, New Zealand

CHRISTOPHER J. R. ROBERTSON
Wild Press, PO Box 12397, Wellington 6144, New Zealand

GEOFF K. CHAMBERS
School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington 6140, New Zealand

PETER A. RITCHIE
School of Biological Sciences, Victoria University of Wellington, PO Box 600 Wellington 6140, New Zealand

Abstract Between 2002 and 2011, Buller's albatrosses (*Thalassarche bulleri bulleri* and *T. b. platei*) accounted for 34% of albatross interactions in New Zealand trawl fisheries. However, the relative impact of commercial fisheries on each taxon is uncertain as identifying individuals by morphology is challenging. The aim of this research was to develop a genetic identification method for the 2 taxa. To this end, DNA was isolated from blood samples collected from a total of 73 birds breeding at northern Buller's albatross colonies on the islets of Motuhara and Rangitatahi (total n = 26) and southern Buller's albatross colonies located on Solander Island and North East Island (total n = 47). The degree of genetic differentiation between northern and southern Buller's was estimated by using DNA sequences from a 221 bp segment of the mitochondrial Control Region. The genetic structure between northern and southern groups was high (*pairwise* Φ_{ST} = 0.621, $P < 0.001$). A Bayesian assignment method was used to determine provenance of individuals randomly sampled from fisheries bycatch (n = 97). All bycatch individuals were assigned with maximum probability to either the northern (n = 19) or southern taxon (n = 78; $P = 1.00$). This study demonstrated that sequences from the mitochondrial control region could reliably be used to assign individuals to either northern, or southern breeding populations, and can be used for determining the provenance of seabird bycatch.

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INTRODUCTION

Tube-nosed seabirds (Order Procellariiformes) are regarded as one of the most threatened avian orders (Croxall *et al*. 2012). Their vulnerability to disturbances is largely attributed to long generation times and low fecundity (Arnold *et al*. 2006; Baker *et al*. 2007; Burg 2007; Croxall *et al*. 2012). Among procellariiform families, the albatrosses (Diomedeidae) are considered a high conservation priority (Baker & Gales 2002, Croxall *et al*. 2012). Currently, 15 of 22 species recognized by BirdLife International and the International Union for Conservation of Nature Red List for birds are reported to be globally threatened (BirdLife International 2018). Commercial fishing activities are known to have caused a significant amount of mortality in some albatross populations (Tuck *et al*. 2001; Lewison & Crowder 2003; Zador *et al*.

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*Correspondence: *wold.jana@gmail.com*

2008). However, determining the impact of fishing-related injuries and deaths on particular species or taxon of albatross can be challenging, especially when distinguishing between morphologically similar species. This problem may be compounded by a general lack of identification and taxonomic expertise among on-board observers, or by specimens damaged to such an extent that diagnostic traits are unrecognizable (Edwards *et al*. 2001). Genetic methods have several advantages, which include not requiring well-preserved, whole specimens and returning data that are a stable characteristic throughout the entire life of an individual (Hajibabaei *et al*. 2007; Aliabadian *et al*. 2009).

The use of genetic approaches to estimate the relative proportion of procellariiform taxa found in bycatch has been introduced relatively recently (Edwards *et al.* 2001). In an attempt to standardise a methodological approach the Western & Central Pacific Fisheries Commission adapted an assay based on restriction fragment length polymorphisms (RFLP) of the PCR-amplified mtDNA cytochrome-b region (Inoue *et al*. 2015). This RFLP method was developed specifically to address the need for simple and inexpensive bycatch sampling, as many countries do not have the resources necessary to pursue other, more expensive, methods. However, the RFLP method does not have the level of resolution required to discern differences among sub-species or populations (Inoue *et al*. 2015). Low levels of genetic differentiation mean that distinguishing between isolated populations or breeding colonies within species requires more sensitive genetic methods (*cf* Walsh & Edwards 2005; Abbott *et al*. 2006; Baker *et al*. 2007; Gomez-Diaz & Gonzales-Soli 2007).

Despite the general shift towards high numbers of hypervariable markers using next generation sequencing (see Davey *et al.* 2011), the mitochondrial DNA (mtDNA) control region offers a relatively effective and straightforward method for resolving species- and population-level differences, particularly in groups such as albatrosses where both sexes are known to be philopatric (see Sagar *et al.* 1998; Gauthier *et al.* 2010). Previous studies have used the mitochondrial control region to identify species and discern population level differences within albatrosses (Walsh & Edwards 2005; Abbott *et al*. 2006; Burg 2007). In addition, the control region has been shown to produce concordant results to microsatellite DNA in shy and white-capped albatrosses, suggesting it is a useful marker for a standardized approach (Abbott & Double 2003a; Abbott & Double 2003b).

Buller's albatross (*Thalassarche bulleri*) currently lack a genetic identification method, but consistently appear in the top 5 species recorded by observers in seabird-fishing interactions within the New Zealand Exclusive Economic Zone (EEZ) (Abraham & Thompson 2012; Clemens-Seely *et al*. 2014a, b; Clemens-Seely & Osk Hjorvarsdottir 2016). *Thalassarche bulleri* is currently recognized as 2 subspecies, northern Buller's albatross (*T. bulleri platei*), and southern Buller's albatross (*T. b. bulleri*) by international conservation organizations such as the Agreement on the Conservation of Petrels and albatrosses (ACAP; Double 2006). However, the history of this classification has been contentious. In light of the significant asynchronous breeding patterns, variation of incubation stints, and geographic isolation of colonies, it was proposed that Buller's albatrosses be split into 2 species, *T. platei* and *T. bulleri* (Robertson & Nunn 1998). Some evidence suggests that the sub-species are most likely only distinguishable based on their plumage (see Gill *et al*. 2010; Dickinson & Remsen 2013). However, the reliability of the plumage trait has not been quantitatively validated. In addition, a previous mitochondrial study found little genetic variation between northern and southern Buller's albatross (Chambers *et al*. 2009). It is important to note that these results can be considered provisional, given the small sample size for each taxon (n = 1). Northern and southern Buller's albatross are considered subspecific by international conservation organisations due to the lack of genetic information and morphological similarity, despite significantly asynchronous breeding patterns (see Double 2006).

The uncertainty surrounding the taxonomic status of subspecies within *T. bulleri*, coupled with the cost and difficulty in accessing remote breeding colonies, means there has been limited population monitoring effort for northern Buller's albatross (Fig. 1). Prior to a relatively recent count conducted by Bell *et al*. (2017), the last breeding colony size estimates for northern Buller's albatross took place in 2009 (Fraser *et al.* 2009). Yet, in both instances, only the colonies on the islet of Motuhara were estimated. In contrast, annual colony size estimates are carried out on the Snares, the largest colony of southern Buller's albatross (Sagar 2015; Thompson *et al*. 2016). Furthermore, elucidating the level of genetic differentiation between northern and southern Buller's albatrosses has received limited attention.

The aim of this present study was to use DNA sequences from the mitochondrial DNA control region to determine the phylogeographic structure of northern and southern Buller's albatross populations. The hypervariable section of the mitochondrial DNA control region known as Domain II (CRII) was used because it is reported to have a high level of variation compared with other mtDNA genes in Procellariiformes (Burg &

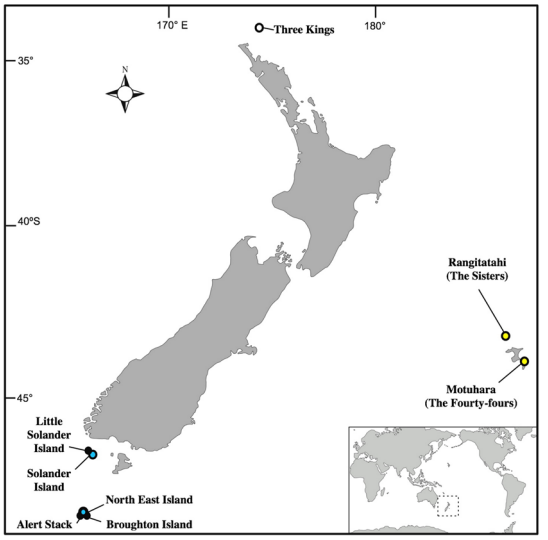

Fig. 1. Locations of northern, and southern Buller's albatross breeding colonies. Sites from which blood samples were collected are denoted by yellow for northern Buller's albatross colonies, while blue denotes southern Buller's albatross colonies. Colonies not represented in this study are either white or black for northern and southern Buller's albatross respectively.

Croxall 2001; Alderman *et al*. 2005; Lawrence *et al*. 2008). The mtDNA sequence data was used to: 1) determine levels of genetic differentiation within and between samples from populations of the northern and southern taxa; and 2) assess whether this genetic marker can be used to assign individual bycatch samples to their population of origin.

MATERIALS AND METHODS

Sampling and DNA extraction

107 blood samples were obtained from chicks and nesting adults between 1996 and 2007 during nest count surveys. Thirty-one samples are from northern Buller's albatross colonies on Motuhara (43°57′42.6″S 175°50′05.5″W) and Rangitatahi (43°34′02.7″S 176°48′25.2″W), while 76 samples are from southern Buller's albatross colonies on North East Island (48°01′30.1″S 166°36′11.4″E) and Solander Island (46°34′19.1″S 166°53′47.8″E). Blood samples were stored in ethanol and kept at -4°C prior to DNA extraction. In addition to the 107 samples of known provenance, liver samples for DNA extraction were harvested from 97 individuals during routine necropsy of bycatch between July 1999 and June 2016. All individuals were collected from within New Zealand's EEZ. These individuals were collected from along the Chatham Rise, the East coast of the North Island, and the eastern, southern and western coasts of the South Island.

Aliquots of blood and tissue samples were digested in extraction buffer (10 mM Tris pH 8.0, 50 mM NaCl, 10 mM EDTA and 0.2% SDS) with 0.5 μg/μL proteinase-K. The DNA was extracted using neutral phenol and phenol-chloroform solutions (Sambrook *et al*. 1989). Total DNA was precipitated using ethanol, dried, and re-suspended in 30 μL TE buffer (10 mM Tris. pH 8.0, 1 mM EDTA). The purified DNA was stored at 4°C prior to analysis and archived at -20°C.

PCR amplification; and DNA Sequencing

The forward primer SPECF1 (5′-AACAGCCTATGTGTTGATGT-3′) and reverse primer GluR7 (5′- CGGGTTGCTGATTTCTCG-3′) from Abbott *et al*. (2005) were used to amplify a 221 bp fragment from the mitochondrial DNA control region II (CRII). All PCRs consisted of approximately 50 ng of DNA, 670 mM Tris-HCl, 160 mM $(NH_4)_2SO_4$, 2.0 mM $MgCl_2$, 0.4 μM of each primer, 0.2 μM of each dNTP, 0.05 U *Taq* polymerase, and 0.4 mg/mL of bovine serum albumin made up to a total volume of 25 μL with ddH_2O. Thermal cycling was performed on an Eppendorf Mastercycler EP Gradient S machine using the following conditions: 2 minutes at 95°C followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 60 seconds at 72°C; a final 72°C extension for 7 minutes. ExoSAP-IT (GE Healthcare) was used to prepare the amplified PCR products (0.5 μL ExoSAP-IT, 0.5 μL of 160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl). An ABI3730 Genetic Analyzer was used to determine the DNA sequence of the amplified products (Macrogen Inc., Seoul, South Korea). Initially, DNA sequences were obtained in both directions. However, after consistently obtaining high quality consensus sequences, only the primer SPECF1 was required for reliable sequencing of the remaining PCR products.

Statistical Analyses

DNA sequences were aligned with CLUSTAL *W* (Thompson *et al*. 1994) and percent pairwise differences between individuals were estimated in Geneious v8.1.7 (Kearse *et al*. 2012). The relative frequency of percent pairwise differences were plotted and the distributions used to investigate the number of fixed differences between groups (Meyer & Paulay 2005). The most appropriate nucleotide substitution model for this fragment of CRII was identified through likelihood ratio tests conducted in jModelTest v2.1.10 (Darriba *et al*. 2012).

DNASP v5.0 (Librado & Rozas 2009) was used to calculate haplotype diversity (h_d), nucleotide

Table 1. Genetic diversity indices and neutrality statistics from CRII sequences from Buller's albatross ssp. (*Thalassarche bulleri* ssp.) of known provenance. n = sample size, S = number of segregating sites, n_h = number of haplotypes, h_d = haplotype diversity, π = nucleotide diversity, K = nucleotide differences, ^{NS} Nonsignificant, * $P < 0.05$, ** $P < 0.001$, *** $P < 0.00001$

Sample information		Diversity Indices					Neutrality Statistics	
Region (and colonies)	n	S	n_h	h_d	π	K	Fu's F_S	Tajima's D
Northern (Motuhara and Rangitatahi)	26	29	21	0.982	0.026	5.726	-12.491***	-0.913
Southern (North East and Solander islands)	47	21	27	0.963	0.013	2.931	-23.531***	-1.243

diversity (π), nucleotide differences (K) and the number of segregating sites (S). ARLEQUIN v3.5 (Excoffier & Lisher 2010) was used to perform an analysis of molecular variance (AMOVA), pairwise Φ_{ST}, and neutrality statistics. In the original paper, Excoffier *et al*. (1992) utilized Φ to refer to the use of genetic distance or haplotype frequency interchangeably in calculating traditional F-statistics. Incorporating genetic distance measures into Φ-statistics is recommended as it not only considers frequency, but also the number of mutational steps among haplotypes (Slatkin 1987; Excoffier *et al*. 1992).

A hierarchical AMOVA was used to assess variability between northern and southern taxa (Φ_{CT}), between breeding colonies within a taxon (Φ_{SC}), and within breeding colonies (Φ_{ST}). Only samples of known provenance (i.e. excluding bycatch) were used to calculate the hierarchical AMOVA. To further investigate differentiation, pairwise Φ_{ST} values were calculated on both the colony and regional level. Motuhara and Rangitatahi represented northern Buller's albatross, while Solander Island and North East Island represented southern Buller's albatross. Finally, to assess the potential influence of variable sample size on levels of differentiation among regions, regional pairwise Φ_{ST} values were calculated again with assigned bycatch. Significance values for the AMOVA, colony and regional pairwise Φ_{ST} comparisons were determined through random permutation procedures (10,000 permutations).

To investigate the population structure of Buller's albatross without a priori assignment to population, data from all samples of known provenance were pooled and genetic groups were identified with BAPS v6.0 (Bayesian Analysis of Population Structure; as per Corander & Tang 2007; Corander et al. 2008; Maltagliati et al. 2010). To identify the maximum number of clusters (K) and to account for the possibility of multiple clusters within regions, 5 replicates were run for each value of K = 1 to K = 6. Analyses were run under cluster with linked loci option as if for 170 reference individuals and for 500 iterations per individual. For assignment of bycatch samples, the sample sets of known provenance and bycatch were pooled into a single group, and a BAPS analysis was repeated under the described conditions. Relationships among mitochondrial haplotypes for all samples were estimated through a median joining haplotype network (Bandelt et al. 1999) as implemented in PopART (Population Analysis with Reticulate Trees; http://popart.otago.ac.nz). This method of network construction builds upon Kruskal's (1956) minimum spanning network algorithm by incorporating a maximum-parsimony heuristic algorithm (Farris 1970) and a bias towards short connections. Haplotype networks were generated for individuals of known provenance and again, bycatch inclusive. Clusters identified with BAPS in samples of known provenance, were then used to visualize the relationships among regionally unique haplogroups with the haplotype network.

RESULTS

Samples of known provenance

From the 107 samples of known provenance, PCR-products were successfully obtained from 73 individuals, of which, 26 (comprising 22 Motuhara and 4 Rangitatahi) were of northern Buller's albatross and 47 (comprising 24 North East Island and 23 Solander Island) were of northern Buller's albatross (GenBank accession numbers MH271445 - MH271614). The failure of many southern Buller's albatross samples was likely due to long-term storage issues, as the quality of the extracted DNA was low.

A 221 bp fragment of CRII was amplified. The combined northern and southern Buller's dataset contained 43 variable sites (S), 48 distinct

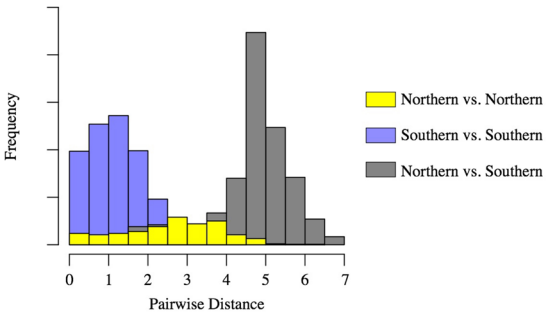

Fig. 2. Frequency of percent pairwise differences within northern (*Thalassarche bulleri platei*) and southern (*T. b. bulleri*) Buller's albatross, and between the 2 taxa.

haplotypes (h) with a haplotype diversity of 0.982 (h_d) and a nucleotide diversity of 0.031 (π). Table 1 shows summary statistics for northern and southern Buller's separately. The most appropriate substitution model identified by jModelTest was Jukes-Cantor (Jukes & Cantor 1969). All calculations which incorporated distance measures were run under this model. Percent pairwise differences among samples of known provenance ranged from 0 to 6.4% within the northern group, 0 to 3.6% within the southern group, and 1.4 to 6.8% between the 2 (Fig. 2). Tajima's D, a statistic used to assess selective or demographic patterns, was negative, but not statistically significant across both northern and southern regions. In contrast, a similar statistic, Fu's F_S, was negative and significant ($P < 0.00001$) for both regions, with values higher within southern Buller's albatross (Fu's F_S = -23.531) than within northern Buller's albatross values (Fu's F_S = -12.491; Table 1).

Colony pairwise Φ_{ST} values demonstrated high levels of differentiation between northern and southern colonies (Table 2), whereas comparisons between colonies within regions were not significant. The largest significant pairwise Φ_{ST} value was found between Rangitatahi and Solander Island (pairwise Φ_{ST} = 0.703, $P < 0.00001$). However, all comparisons between colonies should be viewed with caution due to the small sample sizes, particularly comparisons including Rangitatahi (n = 4).

Regional pairwise Φ_{ST} showed a significantly high degree of differentiation (pairwise Φ_{ST} = 0.618; $P < 0.00001$). The hierarchical AMOVA calculated using Jukes-Cantor corrected distances (Jukes & Cantor 1969) identified no significant regional differentiation and no significant level of differentiation between breeding colonies within a region (Table 3). However, the hierarchical AMOVA indicated high variation within breeding colonies (Φ_{ST} = 0.624, $P < 0.00001$).

Three haplogroups were identified from the samples of known provenance using BAPS (Fig.

Table 2. Colony pairwise Φ_{ST} Pairwise comparison matrix of Φ_{ST} among Buller's albatross colonies using Jukes-Cantor genetic distance (Jukes & Cantor 1969). Motuhara and Rangitatahi are northern Buller's albatross colonies (*Thalassarche bulleri platei*), while Solander Island and North East Island are southern Buller's albatross colonies (*T. b. bulleri*). ^{NS} Nonsignificant, * $P < 0.05$, ** $P < 0.001$, *** $P < 0.00001$.

Colony	Motuhara	Rangitatahi	Solander Island
Motuhara	-		
Rangitatahi	0.0917 ^{NS}	-	
Solander Island	0.6069***	0.7032***	-
North East Island	0.5863***	0.6629**	-0.0094 ^{NS}

Table 3. Analysis of molecular variance. Φ - statistics for CRII from Thalassarche bulleri conducted on geographically structured samples of known provenance. Analyses were run under Jukes-Cantor distance measures (Jukes & Cantor 1969). df = degrees of freedom, NS Nonsignificant, * $P < 0.05$, ** $P < 0.001$, *** $P < 0.00001$.

Source of variation	*df*	Sum of squares	Percentage of variance	Φ-statistics
Among regions	1	109.924	61.069	Φ_{CT} = 0.6038 ^{NS}
Among breeding colonies within regions	2	5.990	1.31	Φ_{SC} = 0.0330 ^{NS}
Within breeding colonies	69	135.317	37.63	Φ_{ST} = 0.6237***

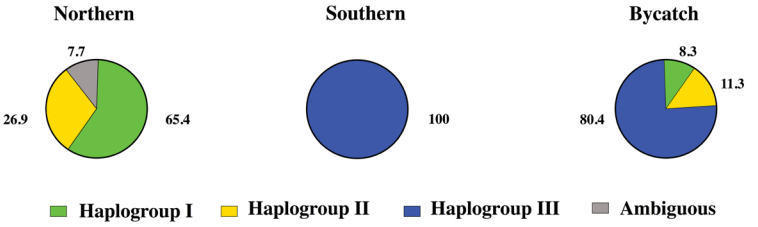

Fig. 3. Proportion of assignment in BAPS for the CRII haplogroups identified in Buller's albatross samples of known and unknown provenance. Proportion of assignment denoted by colour and percentage.

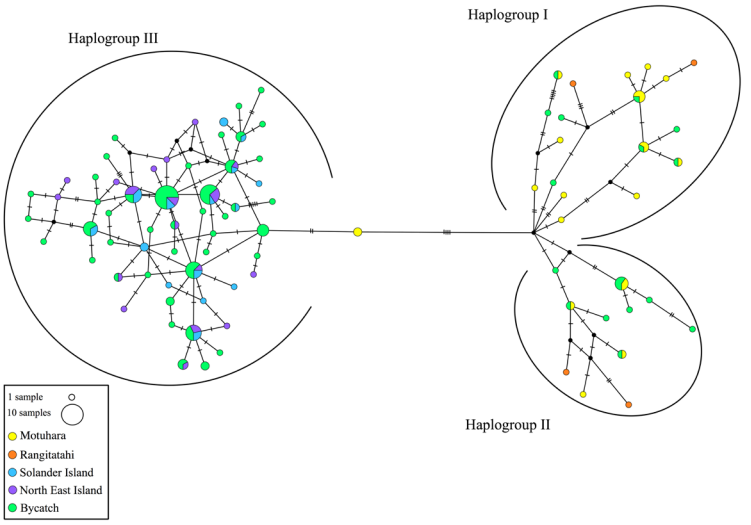

Fig. 4. Median joining haplotype network constructed from DNA sequences obtained from a 221 bp fragment of CRII for 45 northern Buller's albatross (*Thalassarche bulleri platei*) and 125 southern Buller's albatross (*T. b. bulleri*). Each circle represents a distinct haplotype; the relative size of each represents the number of individuals sharing that haplotype. Hash marks indicate 1 mutational change. Each colour is representative of a sample's origins: Site codes are as in Table 1.

3). Haplogroups I & II were exclusive to northern individuals (n = 24) and haplogroup III was exclusive to southern individuals (n = 47). One haplotype, shared by 2 individuals collected from the northern colony, Motuhara, could not be assigned to any 1 of the 3 identified haplogroups ($P < 0.001$), but was positioned directly on the branch separating Haplogroups I & II from Haplogroup III. However, BAPS revealed that this particular haplotype was most similar in sequence to Haplogroup III.

The haplotypes for each region present differing patterns within the network (Fig. 4). Overall the haplotypes observed in northern Buller's albatrosses tended to be at a low frequency compared with the haplotypes observed in southern Buller's albatrosses, and multiple mutational steps separated several northern haplotypes. In contrast, there were generally fewer mutational steps between haplotypes within the southern group, which showed a starburst-like pattern (Slatkin & Hudson 1991). The northern haplotype not assigned to a BAPS haplogroup can be considered an intermediary DNA-sequence type. This haplotype shares relationships with all 3 haplogroups, though fewer mutational steps separate this haplotype from haplogroup III than from haplogroups I & II.

Assignment of bycatch samples

The inclusion of assigned bycatch data with the regional dataset changed the estimates of genetic diversity in both the northern and southern taxa. While nucleotide diversity (π) did not change for the northern taxon, it underwent a small increase from 0.013 to 0.014 in the southern taxon. In contrast, haplotype diversity (h_d) decreased slightly in both groups from 0.982 to 0.976 for northern Buller's albatross and from 0.963 to 0.962 for southern Buller's albatross. Tajima's D was not significant for the northern taxon, but significant for the southern taxon. Fu's F_S remained significant for both groups and increased from -12.491 to -20.393 in northern

Table 4 Genetic diversity indices and Neutrality Statistics from CRII sequences from Buller's albatross of known provenance and assigned bycatch. n, sample size; *S*, number of segregating sites; n_h, number of haplotypes; h_d, haplotype diversity; π, nucleotide diversity; *K*, nucleotide differences, ^{NS} Nonsignificant, * $P < 0.05$, ** $P < 0.001$, *** $P < 0.00001$.

Sample Information		Diversity Indices					Neutrality Statistics	
Region	n	*S*	n_h	h_d	π	*K*	Fu's F_S	Tajima's D
Northern	45	36	31	0.976	0.026	5.769	-20.393***	-1.026^{NS}
Southern	125	36	57	0.962	0.014	3.109	-26.354***	-1.613*

Buller's albatross and from -23.531 to -26.354 in southern Buller's albatross (Table 4). When bycatch samples were assigned to their region of origin and included in the calculation of regional pairwise Φ_{ST}, values remained significant and increased from 0.618 to 0.621 ($P < 0.00001$).

The 3 haplogroups identified in samples of known provenance were retained after the inclusion of the 97 bycatch samples to the BAPS analysis. All 97 individuals were assigned to 1 of these 3 haplogroups with maximum probability ($P = 1.00$). Of the 97 samples of unknown provenance, 19 belonged to haplogroups identified in northern Buller's albatross and the remaining 78 belonged to the southern Buller's albatross haplogroup (Fig. 3).

DISCUSSION

The mitochondrial DNA control region was able to successfully assign all bycatch samples to subspecies (Fig. 3). Of the bycatch, 20% were assigned to haplogroups associated with the northern Buller's albatross and the others were all part of the southern group. Despite the lack of a clear 'barcoding gap' (Fig. 2), the haplotype network and BAPS analyses showed that northern and southern Buller's albatrosses are comprised of distinct mitochondrial lineages (Figs. 3 & 4). The haplotype network patterns and neutrality statistics suggest that the northern Buller's albatross population has historically been demographically stable. In contrast, the starburst pattern of haplotypes and negative neutrality statistics suggests that the southern Buller's albatross has undergone a population expansion. The suggestion of an expanding southern population is also supported by the lower haplotype diversity and nucleotide diversity, and number of nucleotide differences, both when including and excluding the bycatch samples.

The level of DNA sequence diversity measured here at the CRII locus is similar to the levels of nucleotide and haplotype diversity reported for CRI section of the mtDNA sampled from other procellariiform species (Burg & Croxall 2001; Alderman *et al*. 2005; Lawrence *et al*. 2008). The lower levels of diversity and the significantly negative neutrality values (Table 1) within southern Buller's albatross could be explained by either: 1) southern Buller's albatross were founded during a range expansion of northern Buller's albatrosses; or 2) southern Buller's albatross experienced a recent population bottleneck (Tajima 1989; Fu 1997). Past glaciations are believed to have largely influenced the contemporary population structure in Southern Ocean seabirds. As a result, speciation through range expansion is believed to have given rise to many of the taxa we see today and may play a role in the differentiation observed within Buller's albatrosses (see Munro & Burg 2017 for review).

The median joining network of southern Buller's albatross haplotypes has a starburst-like pattern, which is consistent with the suggestion of a recent population expansion (Slatkin & Hudson 1991). When population size increases, the effect of genetic drift is reduced and mutations are more likely to persist. During the initial expansion of a population, haplotypes occur at a relatively low frequency and are only separated by a few mutational steps as seen in southern Buller's albatrosses (Fig. 4). In contrast, the pattern of a historically stable population is a haplotype network with many divergent lineages, which was a feature of the northern Buller's albatross sample. In the case of the northern Buller's albatross, the median-joining network is made up of many distinct haplotypes that occur at a relatively low to moderate frequency and are separated from each other by a relatively large number of mutational steps. The contrasting patterns of genetic diversity in northern and southern Buller's albatrosses support the suggestion that there has been a lack of gene flow between the two groups, and they are most likely 2 demographically uncoupled populations.

Genetic Structure

The level of DNA sequence divergence between northern and southern Buller's albatross haplotypes

varied from 1.4–6.8%. Because of the wide range of variation within each group, there was no clear distinction between the differences within and between the 2 taxa (Fig. 2). The high levels of sequence diversity within northern Buller's albatross may have confounded AMOVA measures of divergence between the 2 regions.

Northern and southern Buller's albatross exhibit similar levels of haplotype diversity, and the Fu's F_S analysis showed that there was an excess of rare haplotypes in both groups. Regional pairwise Φ_{ST} values showed high levels of differentiation between northern and southern Buller's albatrosses (regional pairwise $\Phi_{ST} = 0.62$). Pairwise Φ_{ST} values are consistently high, and significant, between colonies sampled in different regions. In contrast, pairwise Φ_{ST} comparisons between colonies within a region are not significant (Table 2). However, these high values may be misleading. The family of F-statistics was originally developed for biallelic nuclear genes and its application to mitochondrial data may produce inflated values due to the mitochondrial genome being haploid and maternally inherited (Slatkin 1987; Avise 2000). Nevertheless, these regional and colony level comparisons clearly indicate that northern and southern Buller's albatrosses are genetically differentiated.

The hierarchical AMOVA (Table 3) showed that differentiation between regions, and between colonies within each region, was not significant. These AMOVA results are partially consistent with pairwise colony comparisons (Table 2), which indicate that colonies within a region are not differentiated based on mtDNA markers. The lack of genetic structure among colonies within a region found in this study is consistent with a previous analysis of microsatellite DNA from southern Buller's albatross (van Bekkum *et al*. 2006).

Interestingly, the hierarchical AMOVA indicated that variation was significant at the colony level (Φ_{ST} = 0.62, $P < 0.00001$). However, the variance within colonies (Φ_{ST}) is roughly equal to that between taxa (Φ_{CT}; Table 3), which has potentially skewed the null distribution of the hierarchical AMOVA to favour differentiation within colonies (Φ_{ST}) over differentiation between taxa (Φ_{CT}) or among colonies within a taxon (Φ_{SC}). Consequently, the hierarchical AMOVA did not distinguish between variation at the colony and regional level. This has resulted in no statistically significant differentiation between taxa, despite the 2 regions not sharing haplotypes. This was a contrast to pairwise Φ_{ST} comparisons, whereas colonies between taxa resulted in very high levels of differentiation (i.e. Motuhara vs. Solander Island; Table 2).

Regional differentiation is further supported by the isolation of haplogroups to either the northern or southern groups using BAPS. Despite the overlap in inter- and intra-group pairwise differences between northern and southern Buller's albatross (Fig. 2), the 2 taxa formed distinct groupings when graphed as a haplotype network (Fig. 4). The ambiguous haplotype from Motuhara sits between the 2 groups, and is identified by BAPS as more closely related to the southern haplogroup. This haplotype may be a relic of incomplete lineage sorting between the 2 geographically separate populations. Given that no bycatch samples shared the ambiguous Motuhara haplotype, and the high level of variation observed within the northern population, this haplotype is probably relatively rare within northern Buller's albatross. Increased sampling effort would provide better support for this conclusion. Furthermore, the addition of nuclear DNA loci would help determine the bi-parental estimates of genetic connectivity between populations and facilitate testing for the effects of recent population bottlenecks using a range of independent loci (Neigel 1997; Waples & Gaggiotti 2006).

Asynchronous breeding seasons of the northern and southern groups could be an important factor limiting gene flow. Northern Buller's albatross return to colonies and begin breeding 2 to 3 months prior to southern Buller's albatross (Robertson & Sawyer 1994; Robertson in prep.). Friesen *et al*. (2007) noted a strong correlation between significant levels of genetic differentiation and geographically isolated non-breeding areas for a number of seabird taxa. While the asynchronous breeding season between the 2 groups is well documented (Robertson & Sawyer 1994; Sagar & Warham 1998; Fraser *et al*. 2008), any geographical difference in nonbreeding at-sea areas between northern and southern Buller's albatrosses is currently unknown.

The at-sea distribution of southern Buller's albatross has been documented in the Tracking Ocean Wanderers Project (BirdLife International 2004). This database is a collection of studies which have implemented platform terminal transmitters (PTT's) and GPS tracking data for the southern group and used as a proxy for the distribution of all Buller's albatrosses as a whole (Sagar & Weimerskirch 1996; Stahl & Sagar 2000a, b; Broekhuizen *et al*. 2003; BirdLife International 2004). However, data for the at-sea distribution of northern Buller's albatross are limited to 2 tracking records collected between the 10-15 November, 2008 (BirdLife International 2004) and do not properly represent the distribution of the northern taxon. The genetic differentiation observed in this study and the reports of asynchronous breeding seasons between regions, may indicate that the 2 taxa might have distinct distributions during the breeding and nonbreeding seasons. The behavior of individuals from different populations at-sea may

have significant implications for the types of threats they encounter.

Assignment of Bycatch

The idea that genetic differentiation could be used to assign bycaught procellariiform individuals to their population of origin was first proposed by Edwards *et al*. (2001) and has since been developed for a number of seabird species. For example, Burg (2007) and Militao *et al*. (2014) utilized frequency-based assignment methods alongside other metrics, such as nuclear markers, morphology, or stable isotope analysis to differentiate recently diverged species and subspecies.

In the present study, the performance of a 221 bp fragment of CRII in assigning the provenance of individuals was assessed. This was done by characterizing patterns of genetic variation within and between northern and southern Buller's albatrosses. Two putative northern haplogroups and one southern haplogroup were identified using BAPS and these were visualized on a haplotype network (Figs. 3 & 4). All 97 bycatch samples were unambiguously assigned to either the northern or southern haplogroups. Nineteen were identified as northern Buller's albatross and the remaining 78 as southern Buller's albatross.

This study has been able to assess the relative proportions of northern and southern Buller's albatross in fisheries bycatch. Approximately 20% of the bycatch samples were northern Buller's albatross, which highlights the need for awareness of the importance of monitoring of northern Buller's albatross colonies and the need to produce better estimates of incidental mortality. Expanding the northern and southern Buller's albatross genetic reference dataset is integral to improving the range of reference DNA sequences. Increasing the sample size will give a better estimate of genetic diversity within each taxon and increase the certainty of assigning a bird to its population of origin. This information could be used as part of population studies by assessing the role of seasonality and identifying overlaps between commercial fisheries and the at-sea distribution of northern and southern Buller's albatross.

This study reported a DNA-based method for identifying northern and southern Buller's albatross. Sequencing of the mitochondrial control region can be used to assign individuals to their natal colony, even if a specimen is in poor physical condition. A method for accurately identifying northern Buller's albatross will enable the impact of incidental mortality on the northern Buller's albatross to be properly assessed. However, there are still gaps in the estimates of population size and vital ecological statistics for the northern taxon. Nevertheless, this genetic approach could be expanded to include a number of other threatened seabird taxa that are comprised of isolated populations to better assess the impacts of fisheries related mortality.

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