Notornis, 2009, Vol. 56: 82-94
0029-4470

Phylogenetic analysis of the 24 named albatross taxa based on full mitochondrial cytochrome *b* DNA sequences

GEOFFREY K. CHAMBERS*
CASSIDY MOEKE
RYAN STEEL
School of Biological Sciences, Victoria University of Wellington, PO Box 600 Wellington, New Zealand

JOHN W.H. TRUEMAN
School of Botany and Zoology, Australian National University, ACT 2000, Australia

Abstract A stable evidence-based taxonomy is a critical requirement for the effective future conservation of the albatrosses. Recently published partial molecular phylogenies are in broad agreement with respect to the structure of the evolutionary tree for most named taxa, but the analytical methods used to create them have been seriously criticised and they must be considered provisional at best. A further problem is that their authors reach startlingly different conclusions regarding the numbers of taxa which should be recognised as species; 13 *vs.* 24. Here, we attempt to resolve this situation by supplying full length mitochondrial cytochrome *b* data presently missing for 2 taxa, carrying out thorough phylogenetic analyses meeting the requirements of published prescriptions and taking into full account other sources of new molecular data and contemporary opinions on albatross nomenclature and the status of taxa. We provide general support for the published trees and critically evaluate claims regarding how many taxa represent full species. Some genetic distances between pairs of taxa are so small that considerable weight of alternative evidence is required to support any decision leading to a recommendation to split them. We note that the empirical boundary between consensus and controversy falls at or around 1% DNA sequence divergence and further that few, if any, commentators recognise taxa that are separated by less than 0.1% as being valid species.

Chambers, G.K.; Moeke, C.; Steel, R.; Trueman, J.W.H. 2009. Phylogenetic analysis of the 24 named albatross taxa based on full mitochondrial cytochrome *b* DNA sequences. *Notornis 56(2):* 82-94.

Keywords albatross; phylogeny; molecular systematics; cytochrome *b* DNA

INTRODUCTION

Albatrosses are a long-lived charismatic component of the oceanic avifauna. They presently face many threats from pollution of the marine environment and the activities of international fishing fleets (Croxall & Gales 1998, Mills & Ryan 2005). These problems are compounded by their intrinsically slow rate of reproductive increase, with most species raising only 1 chick every year or every 2 years, and losses due to disruption of breeding pairs. The situation is dire, but not impossible, and a new international agency, the Parties to the Agreement on the Conservation of Albatrosses and Petrels (ACAP) has been set up to oversee the situation. This body recognises that a key factor in the effective conservation management of these species is a good understanding of species descriptions and distributions (ACAP 2005). As a first step towards this goal they have set up a Taxonomy Working Group (TWG) to critically review the published data and make recommendations to the ACAP via the annual meeting of their Advisory Committee (AC).

Albatross systematics has a long and complex history (Christidis & Boles 1994, 2007, OSNZ Checklist Committee 1990, 2010). During the last 300 years biologists have produced formal

Received 22 Jul 2009; accepted 6 Nov 2009
Corresponding author: *geoff.chambers@vuw.ac.nz*

descriptions of more than 80 separate taxa (ACAP 2009). Until recently these were entirely based on morphology, occasional reports of field observations and behavioural anecdotes. In recent times, it has become conventional for the entire assemblage to be bundled into just 2 genera (Jouanin & Mougin, 1979); *Phobetria* (sooty albatrosses) and *Diomedea* (everything else) with variable numbers of species and subspecies (e.g. Sibley & Monroe 1990 list 14 species). The application of molecular methods began about 10 years ago with an examination of those on this list (Nunn *et al.* 1996) and later expanded to 22 taxa and included most of the recognised subspecies (Nunn & Stanley 1998). This work was based on examination of full length nucleotide sequences from mitochondrial cytochrome *b* genes and was incorporated into a synthetic proposal for all 24 named taxa entitled '*Towards a new taxonomy for albatrosses*' (Robertson & Nunn 1998).

The operative word in the title of this work is '*towards*' and reflects the intent of the authors that this be regarded as a *working hypothesis*, rather than an exhaustive evaluation of all available evidence. In short, all 24 taxa were raised to species level. Despite the caveat above, this suggestion has been generally well received, but not universally adopted in its entirety (Brooke 2004, Onley & Scofield 2007), i.e., most authors accept most, but not all, of the splits while keeping their own counsel regarding which ones. Perhaps the strongest feature of the Robertson and Nunn (1998) model is their re-establishment of 4 genera (after Alexander *et al.* 1965); *Phobetria* and *Diomedea* are retained, but the latter now restricted to just the large royal and wandering albatrosses, while 2 new genera *Phobastria* (containing all Northern Hemisphere taxa) and *Thalassarche* (smaller albatrosses sometimes commonly known as 'mollymawks') are established. These generic names have been widely adopted by recent authors (Tickell 2000, Brooke 2004, Christidis & Boles 2007, Onley & Scofield 2007, OSNZ 2010). The strongest critics of their model (Penhallurick & Wink 2004) accepted the 4 genera, but lumped the taxa into just 13 species. They based this decision on their interpretation of a '*Multidimensional Species Concept*' (MDSC) after Mayr (1996), as applied to their novel phylogenetic analysis of the Procellariformes. This study (and to some extent those carried out by their predecessors) has been extensively criticised in turn (Rheindt & Austin 2005). One key point is that the MDSC had simply been reduced to a '*barcoding*' (see Methods) decision process based on uncorrected nucleotide sequence divergence, where only taxa separated by greater than 1% divergence were recognised as species. Uncritical application of this view in the mistaken belief that it reflects a consensus can lead to confusion. For instance, Lindsay (2008) tries to adopt (p.114) both the 24 and 13 taxon models simultaneously via a covert *'superspecies'* approach.

Overall, the short comings of the present situation can be summarised:

(i) The cytochrome *b* dataset is incomplete with only 22 of 24 described taxa represented.
(ii) The 2 leading published phylogenetic analyses are congruent, but independently inadequate.
(iii) There has been inappropriate and/or inconsistent adoption and use of species concepts in these previous reports.
(iv) Investigators have missed the opportunity to incorporate new molecular data from other loci – notably mtDNA control region sequences from shy albatross (Abbot & Double 2003a,b), black-browed, and wandering albatrosses (Burg & Croxall 2001, 2004, respectively).

Here we attempt to fill the gaps in (i) by supplying mitochondrial cytochrome *b* data for the 2 missing taxa: white-capped (*T. steadi*) and northern (= Pacific) Buller's albatross (*T. bulleri platei*; see Methods for recent changes to common and scientific names for taxa). We address (ii) by carrying out a thorough phylogenetic analysis of the 24 taxon dataset intended to satisfy all previously published criticisms and supply a full matrix of genetic distances between taxa. Finally, we consider the taxonomic ranking of the terminal branches in our tree(s) to the extent that this gene is informative about those rankings and consider opinions based on the new data in (iv) in the light of published commentary on (iii) from ACAP and others in the sources above.

METHODS

Tissue samples and DNA extraction

Extracts were isolated from frozen liver samples taken from by-catch autopsies and provided by C.J.R. Robertson with reference numbers; 54014 for *T. steadi* and 54245 for *T. platei*. The DNA extracts were originally made by van Bekkum (2004) using methods described in van Bekkum *et al.* (2004) and stored frozen at -80°C.

PCR amplification and DNA sequencing

The mitochondrial cytochrome *b* target was amplified from template DNA (5 μL each reaction) using the L14863/H16065 primer pair under the reaction conditions and thermal cycling regime described by Nunn *et al.* (1996). The quality and quantity of dsDNA amplification products were estimated by agarose gel electrophoresis. Products from reaction giving clean single bands of the expected size (~1200 bp) were purified using Roche High Pure kits according to the manufacturer's instruction. Fluorescent dideoxynucleotide cycle DNA sequencing services were provided by the Allan Wilson Centre at Massey University, Albany Campus and data returned as electronic files.

Table 1. Albatross taxa examined in this study with names after ACAP (2009) including subspecies. * The data for these taxa are new to this study.

Common Name	Scientific Name	GenBank #
Genus *Phoebastria*:		
Short-tailed albatross	*P. albatrus*	U48952.1
Waved albatross	*P. irrorata*	U48951.1
Laysan albatross	*P. immutabilis*	U48949.1
Black-footed albatross	*P. nigripes*	U48950.1
Genus *Diomedea:*		
Southern royal albatross	*D. epomophora*	AF076049.1
Northern royal albatross	*D. sanfordi*	U48946.1
Tristan albatross	*D. dabbenena*	U48947.1
Antipodean albatross	*D. a. antipodensis*	AF076047.1
Gibson's albatross	*D. a. gibsoni*	AF076050.1
Amsterdam albatross	*D. amsterdamensis*	U48948.1
Wandering albatross	*D. exulans*	AF076048.1
Genus *Phoebetria*:		
Sooty albatross	*P. fusca*	U48942.1
Light-mantled albatross	*P. palpebatra*	U48943.1
Genus *Thalassarche*:		
Indian yellow-nosed albatross	*T. carteri*	AF076091.1
Atlantic yellow-nosed albatross	*T. chlororhynchos*	U48944.1
Grey-headed albatross	*T. chrysostoma*	U48954.1
Campbell albatross	*T. impavida*	AF076093.1
Black-browed albatross	*T. melanophris*	U48955.1
Buller's albatross	*T. b. bulleri*	U48945.1
Pacific albatross	*T. b. platei*	EU024821*
Chatham albatross	*T. eremita*	AF076092.1
Salvin's albatross	*T. salvini*	AF076094.1
Shy albatross	*T. cauta*	U48953.1
White-capped albatross	*T. steadi*	EU024820*

Data analysis

The DNA sequence files were checked carefully by eye to eliminate misassigned bases and full sequences assembled using DNAStar Inc Lasergene software package. Further quality assurance checks were made by translating the ORFs into protein sequences and making sure that they corresponded to typical cytochrome *b* protein sequences containing neither stop codons nor non-conservative amino acid substitutions. These consensus sequences were further checked for close nucleotide sequence matches to their close relatives (see below). These 2 sequences have been deposited in GenBank with acquisition numbers (EU024820 and EU024821). They were then combined into an extended data set with the sequences for other taxa as used by Penhallurick & Wink (2004) but independently downloaded from the NCBI database (http://www.ncbi.nih.gov/). We also downloaded outgroup sequences for giant petrel (*Macronectes giganteus*) and grey petrel (*Procellaria cinerea*). However, since we discovered that both of these taxa are now represented by 2 slightly divergent cytochrome *b* sequences we imported both for each respectively: AF076060* + U48941 for *M. giganteus* differ by 0.88% and AP009191 + U48940* for *P cinerea* differ by 0.35%. Those marked (*) were the ones used in Penhallurick & Wink (2004). A full list of the taxa examined is given in Table 1.

First, we constructed a Neighbour Joining tree with PAUP* 4.0b10 (Swofford 2002) using all 28 sequences and assembled a matrix of uncorrected 'p' distances followed by bootstrap repeats with a 1000 pseudoreplicates to obtain bootstrap support values. Next, we carried out a cladistic parsimony analysis in PAUP* 4.0b10 with characters unordered and using a branch and bound search which returned just 1 shortest tree. The procedure was repeated with 1000 pseudoreplicates to obtain bootstrap values and then rerun with tv/ts weightings of 2:1 and 3:1 which yielded the same shortest tree. Finally, we ran a maximum likelihood (ML) analysis in PAUP* 4.0b10 using the model of nucleotide sequence evolution (K81 + I + G) recommended by ModelTest V 3.06 software (Posada & Crandall 1998) under the Aikake Information Criterion (AIC). The procedure was repeated with 100 pseudoreplicates and fast search setting to obtain bootstrap values. We note in

passing that preliminary analyses using TVM + G, JC and HKY85 models of evolution gave the same result. Further details of analytical parameters and methods are given for the tree diagram in Figure 1 and Table 2. Finally, we analysed the data using the GTR+I+G model with all parameters estimated from the data, as implemented in the program GARLI (Zwikl 2006), to obtain both a best-fit maximum likelihood tree and branch support values based on a 1000 pseudoreplicate bootstrap run.

We did not perform extensive Bayesian analyses principally because the 3 independent methods above had already returned identical trees showing that the tree estimate for these data is insensitive to tree estimation procedure. There are also other technical and practical reasons for this decision: 1) Bayesian tree estimation with flat priors on the trees reduces to likelihood-weighted survey of reasonably likely trees and as such provides little information beyond the conventional ML procedure, and 2) a conventional ML tree can be computed from our data in reasonable time and hence there is no need for Bayesian tree estimation as a shortcut. However, we did run quick checks using MrBayes (Hulsenbeck *et al.* 2001) at default settings under the F81 and HKY + I + G models of evolution and demonstrated that both return the same tree identical to the one produced by ML and other methods of analysis.

Taxonomic approach

We advocate a 'total evidence' approach which amounts to diagnosis of taxa as reliably differentiated as based on all available published information. The sections below outline our position on the use of molecular distance data and phylogenetic separation in relation to systematic and nomenclatural considerations.

DNA barcodes

This is a relatively new initiative. An international consortium is building an open access '*Tree of Life*' based on biological classification using short DNA sequences from a standardized region of the genome (e.g. Stoeckle 2003, Edwards 2005). The preferred target is a 648-bp region of the mitochondrial cytochrome *c* oxidase subunit I gene (COI). These sequences are used to create a public library of '*DNA barcodes*' linked to reliably identified and fully described specimens.

The leading assertion underlying this programme is that there is general concordance between mtDNA gene trees and species trees. This is based partly on empirical observations and partly on assumptions based on extrapolation. Hence, the organisers note that genetic variation forms a discontinuous hierarchy paralleled by taxonomic levels. It is then often taken to be the case that taxa whose COI sequences differ by more than 1% are good species, although this value may differ for some sets of taxa or when other genes (e.g. cytochrome *b)* are used (Newton 2003).

This approach was recently applied to 260 species of North American birds and found to work well (Hebert *et al.* 2004). However, the method is not without its pitfalls and inherent limitations and has attracted a number of influential critics (e.g. Moritz & Cicero 2004). For instance, Hickerson *et al.* (2006) warn that DNA barcoding will fail to discover many animal species. However, the true key diagnostic criterion for declaring a 'barcoding species' is that the range of genetic distances between individuals within the limits of the proposed species should be significantly smaller than those between them and members of other species. This is effectively a form of phylogenetic species concept based on phenetic distance analysis (see Discussion). It is clear that multiple taxon sampling is a minimum requirement to support such claims and any wider application of single cut off values must be taken as both hypothetical and provisional.

Species concepts in birds

A wide variety of species concepts is available for biologists to choose (e.g. Claridge *et al.* 1997). For birds, making the right selection is particularly tricky, since many pairs of taxa become morphologically or behaviourally distinct long before they lose the capacity to interbreed. Historically, the traditional favourites have been morphological, biological and phylogenetic (diagnostic) type concepts, together with their modern counterparts, the General Lineage and Multidimensional Species Concepts (Helbig *et al.* 2002). These authors combine diagnostic characters with evolutionary trajectory as a practical approach to resolving the dilemma of selecting an appropriate concept. Their advice has been followed by ACAP (2006) and by OSNZ (2010).

It is clear that once 2 taxa permanently cease to exchange genes they are on an inevitable pathway leading to genetic, morphological and behavioural divergence leading eventually to reproductive isolation (Randler 2006). But how can an observer tell where particular taxa are actually positioned on this pathway at any given time? Assortative mating in sympatry can be a good clue, as occurs in the black-browed albatrosses on Campbell Is (see Discussion). Allopatric populations provide an extra challenge. For instance, even strong natal philopatry has not lead to divergence of the 2 southern populations of *T. b. bulleri* on The Snares and Solander Is (van Bekkum *et al.* 2004). However, the microsatellite data do show that they are genetically separable from the more distant northern populations of *T. b. platei* (van Bekkum 2004), despite the similarity of their cytochrome *b* sequences reported here.

The problem of avian subspecies is even more difficult (Zink 2004). How can one decide what is and what is not a subspecies when it is so difficult to say what species are in the first place? For birds, such fine distinctions can still be of value, but their reliability tends to depend on the group(s) of taxa under examination. It is well known that particularly well-studied groups may be over divided (Phillimore & Owens 2006).

Albatross nomenclature

The scientific and common names applied to albatross taxa have been in a constant state of flux as each succeeding wave of taxonomic revision breaks upon the field. Luckily, there are many excellent technical guides to this history (Brooke 2004; ACAP 2005, 2006, 2009, Onley & Scofield 2007, Christidis & Boles 2007, OSNZ 2010). Below we review some tricky problems that have recently been resolved.

In the wandering albatrosses ('*exulans complex*'), the taxon formerly known as *D. chionoptera* has now been established as *D. exulans* as first described by Linnaeus (Medway 2006). A new type specimen has been described Schodde *et al.* (2009), and now generally adopted under the common name of wandering albatross, although some authors (Onley & Scofield 2007) still prefer to call it snowy albatross after the common name originally attached to *D. chionoptera.* Also in this group, the Antipodean albatrosses (taxon labels *antipodensis* and *gibsoni*) have been lumped as subspecies. However, the authors above prefer the term New Zealand Albatross for the pair.

In the genus *Thalassarche,* the 2 black-browed albatrosses include the widespread form *T. melanophris.* For many years this has also appeared under the correct spelling *melanophrys* (Brooke 2004) but the *Rules of Zoological Nomenclature* require retention of the original incorrect spelling (ACAP 2009). Next, the taxon label *platei,* used to describe the northern populations of Buller's albatross, must be dropped as the type specimen is reported (Robertson & Nunn 1998) to be a juvenile of the southern form, *T. b. bulleri.* These authors suggest *T. sp. nov.* as a provisional name until an new type specimen can be described. This would be appropriate if this taxon held up as a full species, but since most do not support this view the term *T. ssp. nov.* might be more correct (Onley & Scofield 2006). As Christidis & Boles (2007) and OSNZ (2010) point out, the original observation was not supported by published evidence hence one must resort to *T. b. platei* as we have done here. Finally, the term '*mollymawk*' which was often used to describe the smaller albatrosses that make up this genus has now fallen into disuse.

Two other taxa still remain to be named. These are the distinct lineage of the dark-eyed form of black-browed albatross nesting on the Falklands (Burg & Croxall 2001) and the isolated population of *P. nigripes* described by Edwards *et al.* (2001). We note that the latter probably only represents an isolated inbred population characterised by possession of an otherwise rare mtDNA haplotype. Consequently it is, therefore, unlikely to be recognised even at subspecific level.

The nomenclature system used here follows ACAP practice. OSNZ (2010) has a similar list but lump *T. steadi* and *T. cauta* as subspecies arguing that the former is a Tasmanian population of the latter, the data of Abbott & Double (2003a,b) not withstanding. Birdlife International also follow ACAP but go further and split *antipodensis* from *gibsoni* based on plumage features as mentioned in the text.

RESULTS

The 2 new sequences passed our quality assurance tests and were almost full length, lacking only 21 nt from the 5′ end. These checks showed that our *T. platei* sequence was most similar to that of *T. bulleri,* and similarly, *T. steadi* was closest to *T. cauta*. These observations are consistent with the Robertson & Nunn (1998) model. All tree-estimation methods produced the same best-fit topology with the same relative branch lengths. Figure 1 shows this tree optimised under the K81 + I + G maximum likelihood model. The results under alternative ML models are indistinguishable to the eye. Internal nodes on this figure are numbered sequentially. Almost all internal branches have high bootstrap support across all methods; bootstrap scores are given in Table 2. In the Bayesian analyses, all posterior probablites under the F81 model were ≥ 98% and under the more general HKY + I + G model they generally reflected the same order as the bootstrap values in the ML analyses. For example, the least well supported node 17 has bootstrap <50% and a posterior probability of 62%, with all other values >90% except for node 6 (81%) and node 12 (88%; full figures are available from the authors on application). This demonstrates the now conventional 4-genus arrangement of taxa and matches those reported earlier (Nunn & Stanley 1998; Robertson & Nunn 1998; Penhallurick & Wink 2004) with the addition of the 2 extra albatross taxa and the duplicate outgroups. The matrix of uncorrected 'p' distances between pairs of taxa is given in Table 3.

DISCUSSION

We have produced the first complete mitochondrial cytochrome *b* gene tree for all 24 named albatross taxa based on single accessions (i.e., 1 DNA sequence/ taxon). The tree is robust to estimation methods with high boot strap support for most branches. It can

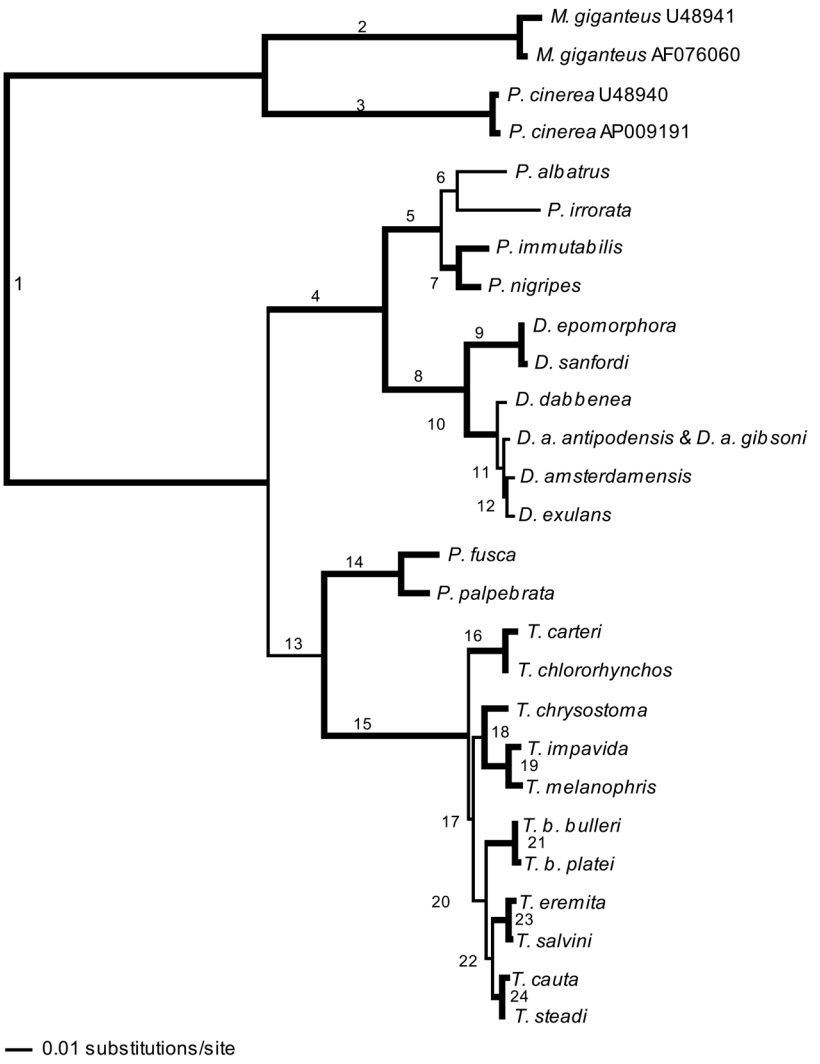

Fig. 1 Best-fit maximum likelihood tree estimated by PAUP and GARLI. The branch lengths are optimised here using the K81 + I + G maximum likelihood model recommended by MODELTEST but all other models give the same tree with virtually identical branch lengths. The ML score (log likelihood) under this model is -4534. Internal nodes are numbered as in the table of bootstrap scores (Table 2) and nodes accepted as valid monophyletic groups on the gene tree are shown by thick lines.

thus be taken as indicating that there is strong signal in the data that may reflect historical relationships between those female lineages which carried these mitochondrial genomes. We acknowledge that gene trees based on mtDNA characters may not correspond exactly to species trees and that gene trees are necessarily dichotomous due to the mode of inheritance of this particular genome. We also acknowledge that we have no information on intra–taxon variation (see our comments on barcoding in the Methods). Furthermore, as the majority of internal branches are short in our tree, caution must guide our interpretations. The 'p' distances of 0.35-0.88 in the 2-sample outgoups illustrate this point well. However, we must be equally careful with respect to the values themselves, since we have no background information to show how closely related or otherwise the 2 pairs of outgroup taxa might be in taxonomic terms.

The 2 additional taxa are placed next to their closest relatives in accord with expectations. The *T. b. platei* sequence shows minimal divergence (0.09%) from *T. b. bulleri*. The *T. steadi* sequence is placed in the 'shy albatross complex' together with *T. cauta*, *T. salvini* and *T. eremita*, but closest to *T. cauta*. This finding is in accord with evidence discussed at length in ACAP (2006) for the *cauta/steadi* pair and in ACAP (2009) for *salvini/eremita.* Hence, we think that it is permissible to make the provisional claim that this particular arrangement of the 24 taxa enjoys general support. Further, we believe that the comprehensive set of phylogenetic analyses that we have carried out are sufficient to satisfy the observations of Rheindt & Austin (2005)

Table 2. Bootstrap scores by node under a range of tree-estimation methods. Nodes judged to have sufficient evidential support in these data to be accepted as delineating a monophyletic group *on the gene tree* are marked * here and are shown in bold on Figure 1.

Node id # As in Fig. 1	Bootstrap score %				Gene-tree nodes with bootstrap support
	NJ with 'p' distances	Unweighted parsimony Branch & Bound	ML- PAUP (K81 + I + G) 'fast' bootstrap	ML -GARLI (GTR+I+G) full heuristic	
1	100	100	100	100	*
2	100	100	85	86	*
3	100	100	100	100	*
4	100	100	91	100	*
5	100	99	98	99	*
6	<50	58	73	71	
7	97	92	92	92	*
8	100	100	96	100	*
9	100	100	99	100	*
10	100	96	88	87	*
11	52	88	75	80	
12	<50	60	55	66	
13	80	77	82	74	
14	100	100	95	97	*
15	100	100	97	98	*
16	100	100	97	99	*
17	55	69	<50	66	
18	87	81	94	93	*
19	100	99	99	100	*
20	96	92	64	93	*
21	100	100	100	100	*
22	87	67	53	73	
23	98	99	95	100	*
24	95	97	72	95	*

on deficiencies in prior analyses of the restricted 22 taxon dataset (Robertson & Nunn 1998, Penhallurick & Wink 2004). The fact that this mtDNA gene tree is robust may help explain why our present results match those reported earlier. But even if this set of relationships may be said to be established beyond reasonable doubt, this is not to say that these will remain unchanged when more extensive data from other mitochondrial targets (e.g. for Control Region as reported by Burg & Croxall, 2001, 2004, Abbot & Double 2003a,b, or for COI as observed by Cassidy, Steel & Chambers, *unpubl. data*) or nuclear loci (e.g. for flanking regions and central repeat units of microsatellite loci as reported by van Bekkum 2004) become available for all 24 taxa, rather than for limited subsets of them. We do note the conventional reservation that although data from further mtDNA targets have the potential to add to the existing phylogenetic signal in the present dataset, the mitochondrial genome should still be regarded as a single locus. Equally, if it should eventually prove possible to increase the number of cytochrome *b* sequences per taxon, then the extended haplotype distributions may blur taxon boundaries. Where this has been achieved for some taxa using other targets such as the mtDNA control region (see below) the

Table 3. The 'p' distance matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 *M.giganteus*	-													
2 *M.giganteus*	0.00875	-												
3 *P.cinerea*	0.11374	0.10936	-											
4 *P.cinerea*	0.11374	0.10936	0.00350	-										
5 *P.albatrus*	0.14261	0.14611	0.15486	0.15311	-									
6 *P.irrorata*	0.15048	0.15223	0.15836	0.15661	0.04374	-								
7 *P.immutabilis*	0.14348	0.14698	0.15398	0.15223	0.03675	0.04724	-							
8 *P.nigripes*	0.14348	0.14698	0.14611	0.14436	0.03500	0.04374	0.01750	-						
9 *D.epomorphora*	0.14961	0.15311	0.15923	0.15748	0.07087	0.06912	0.06737	0.06737	-					
10 *D.sandfordi*	0.14873	0.15223	0.15836	0.15661	0.06999	0.06824	0.06649	0.06649	0.00087	-				
11 *D.dabbenena*	0.14086	0.14436	0.14786	0.14611	0.06387	0.07087	0.06737	0.06212	0.03062	0.03150	-			
12 *D.antip/gibsoni*	0.13823	0.14173	0.14698	0.14523	0.06387	0.06912	0.06562	0.06037	0.03325	0.03412	0.00700	-		
13 *D.amsterdamensis*	0.13998	0.14348	0.14873	0.14698	0.06737	0.07174	0.06737	0.06212	0.03500	0.03587	0.00875	0.00525	-	
14 *D.exulans*	0.13998	0.14348	0.14698	0.14523	0.06212	0.06912	0.06387	0.05862	0.03500	0.03587	0.00875	0.00525	0.00525	-
15 *P.fusca*	0.12598	0.13473	0.13823	0.13648	0.10149	0.10761	0.09886	0.09274	0.09886	0.09799	0.09624	0.09711	0.09711	0.09361
16 *P.palpebra*	0.12598	0.13473	0.13473	0.13298	0.09974	0.10411	0.09624	0.09099	0.09624	0.09711	0.08749	0.08836	0.08836	0.08661
17 *T.carteri*	0.13211	0.13561	0.14436	0.14436	0.11286	0.11374	0.11286	0.10236	0.10586	0.10499	0.09974	0.09711	0.09886	0.09886
18 *T.chlororhynchos*	0.13298	0.13648	0.14261	0.14261	0.11111	0.11199	0.10936	0.09886	0.10586	0.10499	0.10149	0.09886	0.10061	0.10061
19 *T.chrysostoma*	0.13736	0.14086	0.14698	0.14523	0.10849	0.10936	0.10324	0.09449	0.10324	0.10236	0.10324	0.09974	0.10149	0.09974
20 *T.impavida*	0.13998	0.14348	0.14698	0.14523	0.11111	0.10761	0.10761	0.09886	0.10411	0.10324	0.10236	0.10061	0.10236	0.10236
21 *T.melanophris*	0.13736	0.14086	0.14086	0.13911	0.10849	0.11024	0.10499	0.09624	0.10324	0.10236	0.09974	0.09799	0.09974	0.09974
22 *T. b. bulleri*	0.13998	0.14173	0.14436	0.14261	0.11024	0.10936	0.10411	0.09799	0.10149	0.10061	0.10236	0.09974	0.10149	0.10149
23 *T. b. platei*	0.14261	0.14438	0.14698	0.14519	0.11313	0.11231	0.10695	0.10071	0.10430	0.10341	0.10520	0.10253	0.10429	0.10428
24 *T.eremita*	0.14261	0.14523	0.14611	0.14436	0.10674	0.11111	0.10586	0.09974	0.10061	0.09974	0.10061	0.09799	0.09974	0.09974
25 *T.salvini*	0.14173	0.14436	0.14523	0.14348	0.10586	0.11024	0.10499	0.09886	0.09974	0.09886	0.09974	0.09711	0.09886	0.09886
26 *T.cauta*	0.13823	0.14086	0.14261	0.14086	0.10586	0.10849	0.10324	0.09536	0.09974	0.09886	0.09799	0.09536	0.09711	0.09711
27 *T.steadi*	0.13794	0.14062	0.14409	0.14231	0.10590	0.10863	0.10327	0.09526	0.09971	0.09883	0.09793	0.09527	0.09703	0.09702

Table 3. Continued.

	15	16	17	18	19	20	21	22	23	24	25	26	27
15 *P.fusca*	-												
16 *P.palpebra*	0.02100	-											
17 *T.carteri*	0.08311	0.07962	-										
18 *T.chlororhynchos*	0.08136	0.07787	0.00350	-									
19 *T.chrysostoma*	0.07874	0.07699	0.02975	0.02625	-								
20 *T.impavida*	0.08486	0.08136	0.03062	0.02712	0.02012	-							
21 *T.melanophris*	0.08049	0.07699	0.03150	0.02800	0.01925	0.00787	-						
22 *T.bulleri*	0.07699	0.07699	0.03325	0.02975	0.02625	0.02887	0.03150	-					
23 *T.platei*	0.07923	0.07930	0.03472	0.03115	0.02764	0.03032	0.03302	0.00088	-				
24 *T.eremita*	0.08311	0.08049	0.03062	0.02887	0.02362	0.02800	0.02712	0.02012	0.02135	-			
25 *T.salvini*	0.08049	0.07962	0.02975	0.02800	0.02275	0.02712	0.02625	0.01925	0.02047	0.00262	-		
26 *T.cauta*	0.07612	0.07699	0.02887	0.02712	0.02362	0.02712	0.02800	0.01662	0.01782	0.01050	0.00962	-	
27 *T.steadi*	0.07735	0.07651	0.02755	0.02576	0.02225	0.02581	0.02672	0.01513	0.01608	0.01064	0.00976	0.00176	-

resultant data have supported the proposed splits and even finer subdivisions, particularly between allopatric populations of general morphotypes such as the various black-browed albatrosses (Burg & Croxall 2001).

We now turn to the rather more vexed question of how best to label the terminal taxon on each branch: how many of them really merit species rank? Final decisions in this regard go beyond a simple molecular approach. However, an important feature in any such debate is selection of an appropriate species concept to fit the biological situation (see Methods). For relatively simple data, such as those considered here, one tends to be limited to the diagnostic version of the phylogenetic species concept (Cracraft 1983, Mayden, 1997) which emerges as a version of the barcoding paradigm under MDSC (Penhallurick & Wink 2004). The PSC has the well-known potential to inflate species counts as *in extremis* each different DNA sequence might be taken to represent a separate species. Thus, prudent investigators should properly collect multiple DNA sequences to represent each taxon. This must be done to show that the finite variation within the taxon is bounded and well removed from its nearest neighbour in phylogenetic space. Indeed, the same requirement strictly applies equally to barcoding studies in a general sense, but is rarely practical.

The efficient, but not robust, alternative is to establish some cut off value for sequence divergence above which taxa are considered species. The cut off value should be selected with an eye to population sizes, the rate of evolution of the molecular target and the rate of speciation among the taxa under study. In practice, it more often comes down to asking how far diverged well established or 'good' species pairs might be. This looks like a reasonable and objective procedure, but is actually full of all pitfalls. Not least of these is the near truism that all the difficult cases will almost certainly be more closely related than the exemplars. The greater danger is that simple decision rules like a single cut off number have a natural authority to persuade well beyond all reasonable expectations of their actual performance. Nonetheless, we certainly agree that adopting a 1% 'p' distance cut off provides evidence for around 13 organism-level splits. To go beyond this based on cytochrome *b* data alone would be a step too far in our opinion. Rather, it is the case that further splits must be argued on the balance of total evidence and made despite the contrary impression created by the small 'p' distance values. The tree itself is entirely in accord with the proposed relationships between taxa that have emerged based on other evidence.

In their survey work for ACAP, the TWG follows Helbig *et al.* (2002), who use a relaxed version of the

General Lineage Species Concept and starting (as we have here) from the species rich arrangement of Robertson & Nunn (1998) in which pairs were lumped again if there is insufficient published evidence of all types to support the split. We can now proceed to examine their recommendations (ACAP 2006, 2009) in the light of our cytochrome *b* sequence evidence for divergence between pairs of closely related birds. It will be clear from what follows that the general application of a cut off value of 1% divergence may be inappropriate for such a recent radiation. This view is established from consideration of many cases and based on a wide variety of molecular and other evidence.

At the outset we observe that the branches within the genera *Phoebetria* (the 2 sooty albatrosses) and *Phobastria* (4 northern hemisphere albatrosses) lead to well resolved singletons and are regarded as good species by all recent commentators. Our cytochrome *b* trees give no grounds to question this position.

The genus *Diomedea* is divided in our gene tree into clades containing the 2 royal albatrosses and the 5 wandering albatrosses. The former are recognised by TWG in 2007 as *D. epomorpha* and *D. sandfordi* on the basis of well-established morphological and plumage differences. The cytochrome *b* distance is only 0.08% and when considered alongside their potential to interbreed must leave this decision open to review. Indeed, ACAP (2006) lumped the isolated population breeding on Adams Is in the subantarctic as a subspecies of those breeding on the Antipodes Is; *D. a. gibsoni* and *D. a. antipodensis,* respectively. Their decision was made in face of characteristic retention of darker juvenile plumage by adult female Antipodes birds, their distinct foraging patterns (Walker & Elliot 2006) and lack of gene flow between members of these 2 geographically distinct populations (Burg & Croxall 2004). These taxa have identical cytochrome *b* sequences (Nunn & Stanley 1998) and control region sequences, but do show some differentiation at microsatellite loci (Burg & Croxall 2004). The 3 remaining members of *Diomedea* are provisionally recognised as species by ACAP (2006) and other recent publications (Brooke 2004, Onley & Scofield 2007) and reviewed by TWG in 2008 (ACAP 2009). However, their cytochrome *b* distances range from 0.5% between *D. exulans* and *D. amsterdamensis* and 0.9% between *D. dabbenea* and each of the other two. Taken in isolation such values would not support there being more than one good species in this group. Burg & Croxall (2004) support the *exulans/dabbenea* split based on their distinct mtDNA control region lineages.

The genus *Thalassarche* is the most complex clade and consists of 5 subclades. First, the Indian and Atlantic forms of the yellow-nosed albatross (*T. carteri* and *T. chlororhyncus,* respectively) are recognised at species level by TWG in 2007 (ACAP 2009) based largely on breeding distributions and qualitative morphological characters (Marchant & Higgins 1990, Robertson 2002). This pair has also diverged quite recently and their cytochrome *b* distance is only 0.4%. Thus, although the cytochrome *b* tree does not contradict their separation from the other 9 taxa in this genus, it is not sufficient in isolation to justify splitting the yellow-nosed taxon pair.

The second subclade, the grey-headed albatross (*T. chrysostoma*), is a singleton and is accepted by all authors at species level. In our tree, it groups with the 2 black-browed albatrosses (*T. impavida* and *T. melanophris*), but is well separated ('p' distances: 1.8 and 2.0%, respectively). Hence, this particular species hypothesis is supported by the cytochrome *b* data.

The third subclade comprises the 2 members of the *impavida/melanophris* pair. The cytochrome *b* sequences differ by 0.8% between these 2 species but they have been decisively separated by Burg & Croxall (2001) on the basis of independent genetic evidence and can be recognised by the colour of the iris; red in black-browed albatross (*T. melanophris*) and pale (straw) in the Campbell albatross (*T. impavida*). They have recently been reported as breeding in sympatry on Campbell Is, but have distinct calls and mate assortatively (Moore *et al.* 1997), although they are capable of hybridising when the sex ratio of one form is skewed (Moore *et al.* 1997, 2001). This view was supported by TWG in their 2008 report (ACAP 2009).

The fourth subclade involves the 2 forms of Buller's albatross. No recognised authority presently grants them full species status (e.g. Brooke 2004, Onley & Scofield 2007, ACAP 2009) despite the large geographic separation of their breeding sites (>900 km) and observations of Robertson & Nunn (1998) that they breed at different times. Our new data confirm the close relationship between them with a minimal 'p' distance of only 0.09%, a value otherwise strongly indicative that they are conspecific. The nomenclature suggested for this pair is the Buller's albatross (*T. b. bulleri*), breeding on Snares and Solander Is, and northern (or Pacific) Buller's albatross (*T. b. platei*), breeding 2 months earlier at Three Kings Is and at 2 sites in the Chatham Is. Onley & Scofield (2007) suggest *T. b. ssp nov* is preferable to *T. b. platei*, which would be more correct if Robertson & Nunn (1998) had provided published evidence to support their view regarding the type specimen (see Methods). Microsatellite data and assignment tests (van Bekkum 2004) show that these breeding stocks are genetically differentiated and probably no longer interbreed to any significant extent. However, their separation is clearly recent and it has been argued that there are few real grounds even for granting them subspecific

status (ACAP 2006). Our cytochrome *b* data support this latter position.

Finally, the shy albatross complex consists of 4 taxa which deserve most careful attention, since they are very closely related and were formerly classified as members of a single species (e.g. Marchant & Higgins 1990). Our new data cover this group as a whole for the first time. The findings presented here justify the TWG approach of treating them as 2 pairs of 2 taxa: *T. cauta* + *T. steadi* (ACAP 2006) and *T. salvini* + *T. eremita* (ACAP 2009), as shown in Fig. 1. The 'p' distances between pairs reflect this view being around 1% between the pairs but only 0.2% between *T. cauta* and *T. steadi* and 0.3% between *T. salvini* and *T. eremita.* Hence, they should not be grouped into a single species even under the strict Penhallurick & Wink (2004) decision framework. Further, ACAP (2006) presents extensive total evidence arguments for the recognition of *T. cauta* and *T. steadi* at specific level and similarly ACAP (2009) for *T. salvini* and *T. eremita.*

Taking all of the above together it is clear that taxa separated by 'p' distances of 1% or greater are widely recognised at species level. At the other end of the scale there are few reliable sources that split pairs of taxa with 'p' distances equal to or less than 0.1%. Those pairs of taxa with intermediate values must be viewed holistically. Here we see examples of pairs of taxa with 'p' distances as low as 0.2 or 0.3% for which there is other good evidence to show that they satisfy the Helbig *et al.* (2002) criteria for recognition as good species. Newton (2003) collated reports that show many avian species are separated by as much as 6 – 10% cytochrome *b* divergence. However, there are several instances where this value may be as low as 0.1%. This author concludes that "….. *there can be no fixed degree of genetic divergence that defines a speciation event."*

In summary, a mitochondrial gene tree has been prepared for 24 albatross taxa. It includes 22 nominate species whose relationships have been recognised by other authorities with abundant support from other data sources. The list (after ACAP 2009) is provisionally identical with Onley & Scofield (2007) and differs only from Brooke (2004) and OSNZ (2010) by splitting *cauta* and *steadi*. These totals are subject to change as new data emerge. There are 2 remaining enigmatic taxa that we have not had the opportunity to examine here. The first is the unique Falklands Is lineage of black-browed albatross recorded by Burg & Croxall (2001). The second is the isolated Japanese population of *P. nigripes* described by Edwards *et al.* (2001). At first sight the available evidence would seem to support species status for the former, but not the latter. Of course, it would be desirable to have more extensive molecular data, especially from rapidly evolving nuclear loci, for these and indeed all taxa to aid in making such decisions. Recent technical advances by Bakström *et al.* (2008) and Kimball *et al.* (2009) have opened up many new genetic targets for analysis in avian molecular systematics and have already been applied across a wide range of bird orders with considerable success (Hackett *et al*. 2008) albeit associated with obviously high experimental costs. However, in the case of the albatrosses, the rarity of nucleotide variation data reported for chromosomal regions flanking microsatellite loci by van Bekkum (2004), suggests that very large nuclear targets will need to be examined to gain much in the way of resolving power.

We conclude by noting that our study shows comparatively short DNA sequences can give robust gene trees displaying hypothetical evolutionary relationships between taxa. Such trees may be of assistance in recognising conservation priorities, but our experience also shows clearly the dangers inherent in using just one type of information or overly simple and/or unsupported decision rules to determine what is or what is not a good species.

ACKNOWLEDGMENTS

Experimental work in the Chambers Laboratory at Victoria university of Wellington was supported by various Science Faculty Grants including a Summer Research Assistantship for Ryan Steel and an Awhina Scholarship for Cassidy Moeke. The phylogenetic analyses were performed by John Trueman with support from internal resources at the Australian National University, Canberra. The authors are grateful to 2 anonymous reviewers whose comments helped to improve the clarity and presentation of our manuscript.

LITERATURE CITED

ACAP. 2004. Report on the First Meeting of the Parties (MoP1) held 10 – 12 November, 2004 in Hobart, Australia.

ACAP. 2006. Report on the Second Meeting of the Parties (MoP2) held 13 – 17 November, 2006 in Christchurch, New Zealand.

ACAP. 2009. Report on the third Meeting of the Parties (MoP3) held 27 April – 1 May November, 2006 in Bergen, Norway.

Abbott, C.L.; Double, M.C. 2003a. Genetic structure, conservation genetics, and evidence of speciation by range expansion in shy and white-capped albatrosses. *Molecular Ecology 12*: 2953-2962.

Abbott, C.L.; Double, M.C. 2003b. Phylogeography of shy and white-capped albatrosses inferred from mitochondrial DNA sequences: implications for population history and taxonomy. *Molecular Ecology 12*: 2747-2758.

Alexander, W.; Falla, R.A.; Jouanin, C.; Murphy, R.C.; Salmonsen, F.; Voous, K.H.; Watson, G.E.; Bourne, W.R.P.; Kuroda, N.H.; Rowan, M.K.; Sevrenty, D.L.; Tickell, W.L.N.; Warham, J.; Winterbottom, J.M. 1965. The families and genera of petrels and their names. *Ibis* 107: 401-405.

Backström, N.; Fagerberg, S.; Ellegren, H. 2008. Genomics of natural bird populations: a gene-based set of

reference markers spread evenly across the avian genome. *Molecular Ecology 17*: 964-980.
Brooke, M. 2004. *Albatrosses and petrels across the world*. Oxford University Press: Oxford.
Burg, T.M.; Croxall, J.P. 2001. Global relationships amongst black-browed and grey-headed albatrosses: analysis of population structure using mitochondrial DNA and microsatellites. *Molecular Ecology 10*: 2647-2660.
Burg, T.M.; Croxall, J.P. 2004. Global population structure and taxonomy of the wandering albatross species complex. *Molecular Ecology 13*: 2345-2355.
Christidis, L.; Boles, W.E. 1994. *The Taxonomy and species of birds of Australia and its Territories*. RAOU Monograph No. 2, Royal Australasian Ornithologists Union: Melbourne.
Christidis, L.; Boles, W.E. 2007. *Systematics and taxonomy of Australian birds*. CSIRO Publishing: Collingwood.
Claridge, M.F.; Dawah, H.A.; Wilson M.R. 1997. *Species the units of biodiversity.* Chapman and Hall: London.
Cracraft, J. 1983. Species concepts and speciation analysis. *Current Ornithology 1*: 159-187.
Croxall, J.P.; Gales, R. 1998. An assessment of the conservation status of albatrosses. In: *Albatross Biology and Conservation.* (Eds., G. Robertson; R. Gales.) pp. 46-65. Surrey Beatty & Sons: Chipping Norton.
Edwards, J.L. 2005. Consortium for the barcode of life: An introduction. *Genes and Genetic Systematics 80*: 443.
Edwards, S.V.; Silva, M.C.; Burg, T.; Friesen, V.; Warheit, K.I. 2001. Molecular genetics markers in the analysis of seabird bycatch populations. pp. 115-140 in *Proceedings – seabird bycatch: trends, roadblocks and solutions.* University of Alaska: Sea Grant AK-SG-01-01.
Hackett, S.J.; Kimball, R.T.; Reddy, S.; Bowie, R.C.K.; Braun, E.L.; Braun, M.J.; Chojnovski, J.L.; Cox, W.A.; Han, K.-L.; Harshman, J.; Huddleston, C.J.; Marks, B.D.; Miglia, K.J.; Moore, W.S.; Sheldon, F.H.; Steadman, D.W.; Witt, C.C.; Yuri, T. 2008. A phylogenetic study of birds reveals their evolutionary history. *Science 320*: 1763-1768.
Hebert, P.D.N.; Stoeckle, M.Y.; Zemlak, T.S.; Francis, C.M. 2004. Identification of birds through DNA barcodes. *PLoS Biology 2*: 1657-1663.
Helbig, A.J.; Knox, A.K.; Parkin, D.T.; Sangster, G.; Collinson, M. 2002. Guidelines for assigning species rank. *Ibis 144*: 518-525.
Hickerson, M.J.; Meyer, C.P.; Moritz, C. 2006. DNA barcoding will often fail to discover new animal species over broad parameter space. *Systematic Biology 55*: 729-739.
Hulsenbeck, J.P.; Ronquist, F.; Posada, D.; Crandall, K.A. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics *17*: 754-755.
Jouanin, C.; Mougin, J.-L. 1979. Order Procellariformes pp. 48-121 in *Checklist of the birds of the world. Vol I, 2nd ed.* Eds., E. Mayr; G.W. Cotrell. Museum of Comparative Zoology: Cambridge, MA.
Kimball, R.T.; Braun, E.L.; Barker, F.K.; Bowie, R.C.K.; Braun, M.J.; Chojnovski, J.L.; Hackett, S.J.; Han, K.-L.; Harshman, J.; Heimer-Torres, V.; Holznagel, W.; Huddleston, C.J.; Marks, B.D.; Miglia, K.J.; Moore, W.S.; Reddy, S.; Sheldon, F.H.; Smith, J.V.; Witt, C.C.; Yuri, T. 2008. A well-tested set of primers to amplify regions spread across the avian genome. *Molecular Phylogenetics and Evolution 50*: 654-660.
Lindsey, T. 2008. *Abatrosses*. CSIRO Publishing: Melbourne.
Marchant, S.; Higgins, P.J. 1990. *Handbook of Australia, New Zealand and Antarctic birds*. Oxford University Press: Melbourne.
Mayden, R.L. 1997. A hierarchy of species concepts: the denoument in the saga of the species problem. pp. 381-424 in *Species the units of biodiversity*. Eds., M.F. Claridge; H.A. Dawah; M.R. Wilson. Chapman & Hall: London.
Mayr E. 1996. What is a species and what is not? *Philosophy of Science 63*: 262-277.
Medway, D. 2006. The identity of the chocolate albatrosss *Diomedea spadaciea* of Gmelin, 1789 and the wandering albatross *Diomedea exulans* of Linnaeus, 1758. *Notornis 40*: 145-162.
Mills, M.S.L.; Ryan P.G. 2005. Modelling impacts of long-line fishing: what are the effects of pair bond disruption and sex-biased mortality on albatross fecundity? *Animal Conservation 8*: 359-367.
Moore, P.J.; Taylor, G.A.; Amey, J.M. 1997. Interbreeding of black-browed albatrosss *Diomedea m. melanophris* and New Zealand black-browed albatross *T. m. impavida* on Campbell Island. *Emu 97*: 322-324.
Moore, P.J.; Burg, T.M.; Taylor, G.A.; Millar, C.D. 2001. Provenance and sex ratio of black-browed albatross, *Thalassarche melanophris,* breeding on Campbell Island, New Zealand. *Emu 101*: 329-334.
Moritz, C.; Cicero, C. 2004. DNA barcoding: Promise and pitfalls. *PloS Biol 2*, e312 doi:10.1371/journal.pbio.0020312
Newton, I. 2003. *Speciation and biogeography of birds*. Academic Press: Amsterdam.
Nunn, G.B.; Cooper, J.; Jouventin, P.; Robertson, C.J.R.; Robertson, G.G. 1996. Evolutionary relationships among extant albatrosses (Procellariiformes: Diomedeidae) established from complete cytochrome-b gene sequences. *Auk 113*: 784-801.
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.
Onley D.; Scofield P. 2007. *Albatrossses, petrels and shearwaters of the world.* Christopher Helm: London.
OSNZ Checklist Committee – E.G. Turbott, Convener. 1990. *Checklist of the birds of New Zealand and the Ross Dependency, Antarctica, 3rd Ed.* Ornithological Society of New Zealand and Random Century: Auckland.
OSNZ Checklist Committee – B.J. Gill, Convener. 2010. *Checklist of the birds of New Zealand, Norfolk and Macquarie Islands, and the Ross Dependency, Antarctica, 4th Ed.* Te Papa Press: Wellington– *in press.*
Penhallurick, J.; Wink, M. 2004. Analysis of the taxonomy and nomenclature of the Procellariiformes based on complete nucleotide sequences of the mitochondrial cytochrome *b* gene. *Emu 104*: 125-147.
Phillimore A.B.; Owens I.P.F. 2006. Are subspecies useful in evolutionary and conservation biology? *Proceedings of the Royal Society of London Series B 273*: 1049-1053.
Posada, D.; Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics 14*: 817-818.
Randler, C. 2006. Behavioural and ecological correlates of natural hybridisation in birds. *Ibis 148*: 459-467.

Rheindt, F.E.; Austin, J.J. 2005. Major analytical and conceptual shortcomings in a recent taxonomic revision of the Procellariiformes - a reply to Penhallurick and Wink (2004). *Emu 105*: 181-186.

Robertson, C.J. 2002. The scientific name of the Indian yellow-nosed albatrosss *Diomedia carteri. Marine Ornithology 28*: 48-49.

Robertson, C.J.; Nunn, G.B. 1998. Towards a new taxonomy for albatrosses. In: *Albatross Biology and Conservation.* Eds., G. Robertson; R. Gales. pp. 13-19. Surrey Beatty and Sons: Chipping Norton.

Schodde, R.; Gill, B.J.; Scofield, P.; Steinheimer, F.D.; Groth J.G.; Lai J. 2009. Settling application of the name *Diomedea exulans* Linnaeus, 1758, for the wandering albatross by neotypification. *Unpublished manuscript.*

Sibley, C.G.; Monroe, B., Jr. 1990. *Distribution and taxonomy of the birds of the world.* Yale University Press: New Haven.

Stoeckle, M. 2003. Taxonomy, DNA and the bar code of life *Bioscience 53*: 796-797.

Swofford. 2002. PAUP* v4.0b10 www.paup.csit.fsu.edu

Tickell, W.L.N. 2000. *Albatrosses.* Pica Press: Robertsbridge.

Walker, K.; Elliot, G. 2006. At-sea distribution of Gibson's and Antipodean wandering albatrosses and relationships with longline fisheries. *Notornis 53*: 265-290.

Van Bekkum, M. 2004. Microsatellite DNA analysis of breeding behaviour and evolution in Buller's albatross (*Thalassarche bulleri*). MSc Thesis, Victoria University: Wellington.

Zink, R.M. 2004. The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proceedings of the Royal Society of London Series B 271*: 561-564.

Zwickl, D. 2006. GARLI v0.951. Computer program distributed by the author. www.bio.utexas.edu/faculty/antisense/garli/Garli.html.