

## Few genetic differences between Victorian and Western Australian blue penguins, *Eudyptula minor*

JONATHAN C. BANKS\*

R. H. CRUICKSHANK

G. M. DRAYTON†

A. M. PATERSON

Bioprotection and Ecology Division  
PO Box 84  
Lincoln University  
Canterbury 8150, New Zealand

\*Present address: Department of Biological Sciences,  
University of Waikato, Private Bag 3105, Hamilton  
3240, New Zealand. j.banks@waikato.ac.nz

†Present address: Crop & Food Research, Private  
Bag 4704, Christchurch 8140, New Zealand.

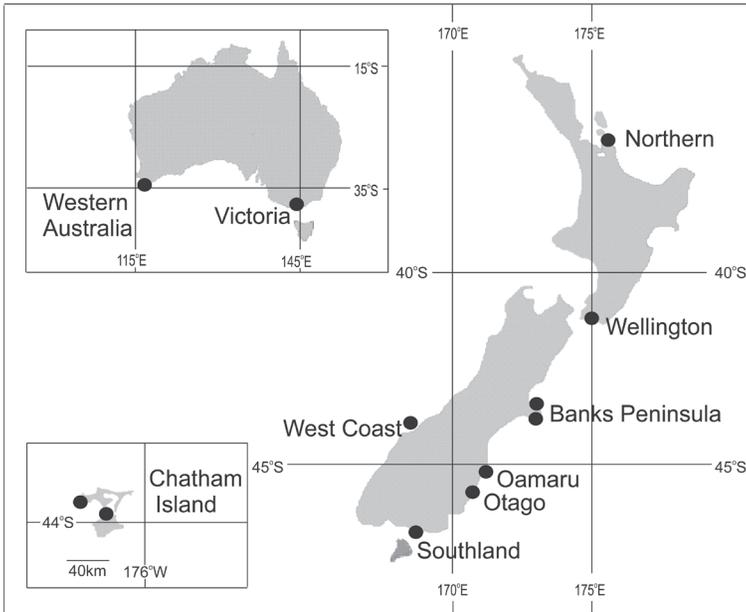
**Abstract** Blue penguins, *Eudyptula minor*, breeding on Penguin Island, Western Australia are considerably larger than other blue penguins in Australia. If genetic isolation is the cause, it may have implications for the conservation status of some blue penguin populations. We compared the sequences of two mitochondrial gene regions (cytochrome-*b* and the control region) from Western Australian blue penguins with other populations of blue penguins from Australia and New Zealand. We found few differences between sequences from Western Australia, Phillip Island, Victoria and Otago, New Zealand, although all three differed considerably from other New Zealand blue penguins. Sequences for the control region from the Western Australian blue penguins and 30 more birds breeding at various Australasian sites provided further support for two major clades within *Eudyptula*; an Australian clade (including Otago) and a New Zealand clade.

**Keywords** control region; cytochrome-*b*; mitochondrial DNA; Sphenisciformes; systematics

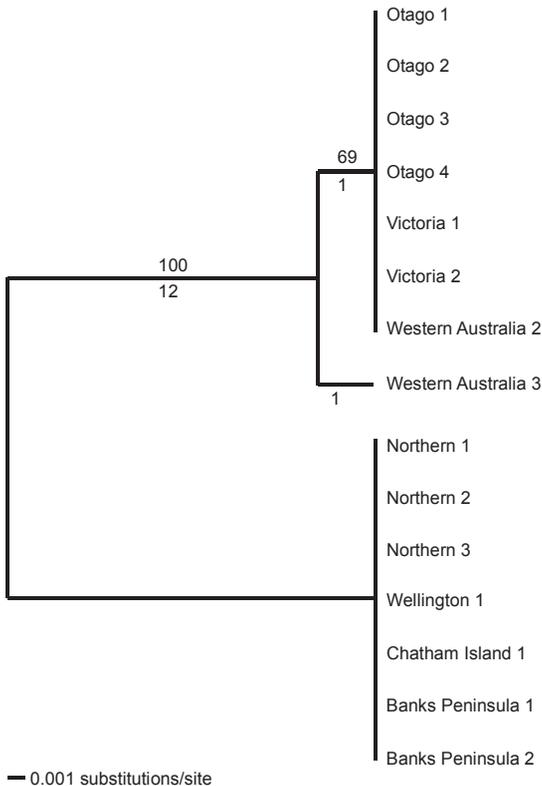
## INTRODUCTION

Blue penguins, *Eudyptula minor* (Forster, 1781), are the smallest living penguins, found in temperate seas around the coasts of New Zealand and the eastern and southern coasts of Australia (Marchant & Higgins 1990). Blue penguin populations have declined in many areas, following human disturbance and the introduction of alien predators (del Hoyo et al. 1992; Challies & Burleigh 2004). Understanding biodiversity and setting priorities for the management of species with declining populations depends crucially on taxonomy (McNeely 2002), because the conservation status of a species is determined from several factors including population size and distribution for example, the IUCN Red List (IUCN 2006). Population sizes and distribution can be known accurately, and conservation management priorities and strategies assigned correctly, only if the taxonomy of a species is correct (for a case study see Daugherty 1990).

The taxonomy of *Eudyptula* is controversial and has been extensively revised since the species was first described as *Aptenodytes minor* J. R. Forster 1781 (Marchant & Higgins 1990). Several subspecies have been distinguished on morphometric data such as bill size, body weight and tarsus length (Kinsky & Falla 1976). Blue penguins from Western Australia are heavier and have longer bills than blue penguins elsewhere in Australia (mean 1390 g compared with 1207 g in Victoria during the non-moulting season, and the size difference between Penguin Island, Western Australia penguins and some of the other blue penguins is greater than the differences between several of the subspecies (Klomp & Wooller 1988). Comparison of random amplified polymorphic DNA from samples collected from birds breeding on Jarvis Bay, New South Wales and Penguin Island, Western Australia found a slight separation between the two populations (Wienecke 1993). Klomp & Wooller (1988) therefore proposed that the larger size of birds from Penguin Island may be due either to the relative isolation of these birds at the northern limit of their distribution, or to a more favourable climate and food supply.



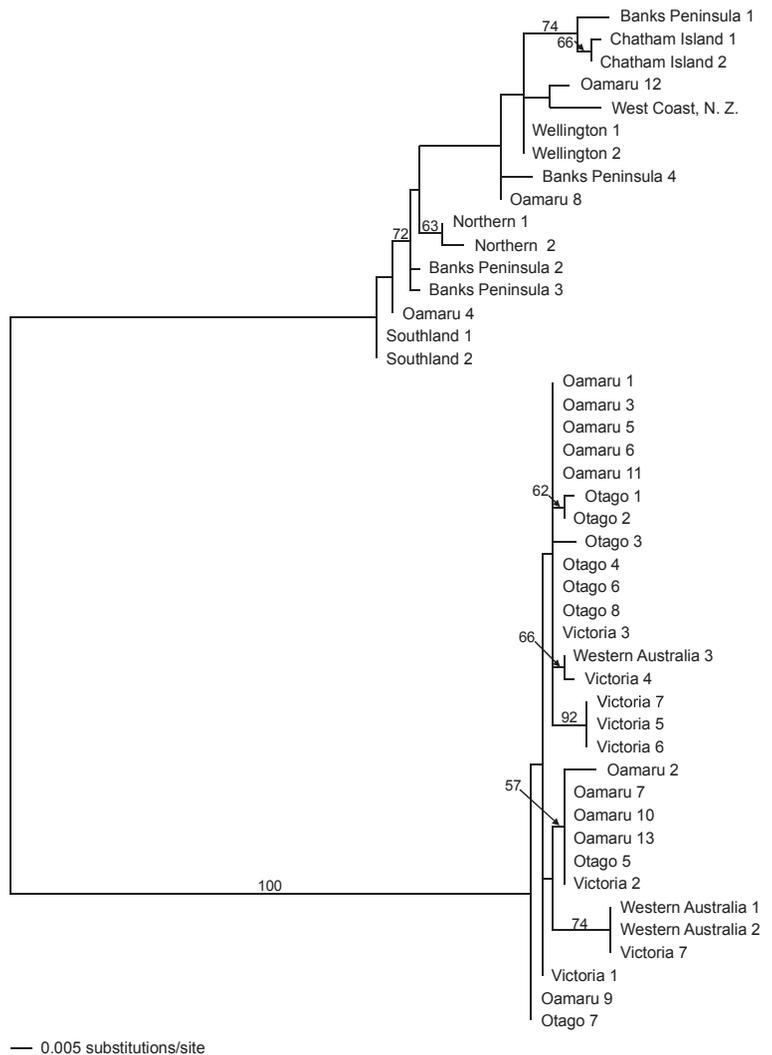
**Fig. 1** Collection localities of blue penguin samples.



In this study, we obtained genetic sequences for two mitochondrial regions, the cytochrome-*b* gene which evolves moderately quickly (Nunn et al. 1996) and the faster-evolving control region (Freeland & Boag 1999; Lambert et al. 2002) to investigate if there are genetic differences between blue penguins breeding on the east and west coasts of Australia. We also compared the western Australian sequences with 12 published sequences and 30 new sequences for the mitochondrial control region from blue penguins breeding in various locations in Australasia, to investigate whether or not sequences from more specimens support the clade structure we previously described (Banks et al. 2002).

**Fig. 2** Phylogenetic tree generated using maximum likelihood analysis (see text for details) of the cytochrome-*b* sequences. Numbers above the lines represent percentage bootstrap support greater than 50% (of 1000 replicates) and numbers below the lines are the number of substitutions in that branch of the cytochrome-*b* sequences. The terminal nodes of the tree are labelled with the collection location and a voucher number for each bird.

**Fig. 3** Phylogenetic tree generated using maximum likelihood analysis (see text for details) of the control region sequences. Numbers above the lines represent percentage bootstrap support greater than 50% (of 1000 replicates). The terminal nodes of the tree are labelled with the collection location and a voucher number for each bird.



**METHODS**

Samples were obtained from birds at locations shown in Fig. 1. DNA was extracted using Qiagen DNeasy kits from 15 mg of muscle tissue or 10 µl of blood. We amplified a portion of cytochrome-*b* (*cyt-b*) gene and the control region (CR) from the mitochondrial genome from total genomic extracts using the methods outlined in Banks et al. (2002).

**Cytochrome-*b***

We obtained the sequence for a 334 nucleotide portion of *cyt-b* from two of the Western Australian specimens (Genbank accession numbers AY194079–

AY194080, available via <http://www.ncbi.nlm.nih>) and compared these two sequences with sequences published previously (Banks et al. 2002), Genbank accession numbers AF468932–AF468944). Using Modeltest version 3.06 (Posada & Crandall 1998), we selected the HKY substitution model (Hasegawa et al. 1985) to conduct a maximum likelihood analysis using the following settings; TI/TV = 13.4876, A = 0.2741, C = 0.3591, G = 0.1309, T = 0.2359, using the heuristic search function.

**Control region**

Because there were few differences between the sequences for *cyt-b* from blue penguins from the

Australian east and west coasts, we chose to focus on sequencing the faster evolving mitochondrial CR for more individuals. We sequenced 406 nucleotides of the CR (Genbank accession numbers AY194081, AY194082, AY194084, available via <http://www.ncbi.nlm.nih>) for three Western Australian specimens and 30 blue penguins from other locations (Genbank accession numbers, AY194083, AY194085, AY194086, DQ017223–DQ017249). We combined these sequences with 12 sequences published previously (Genbank accession numbers, AF468945–AF468956) and used Modeltest version 3.06 (Posada & Crandall 1998) to select the HKY + gamma model (Hasegawa et al. 1985; Yang 1993) for a maximum likelihood analysis with the following settings; TI/TV = 13.8958, Gamma = 0.0623, A = 0.3244, C = 0.2068, G = 0.1856, T = 0.2833, using the heuristic search function.

## RESULTS

The maximum likelihood analysis of the *cyt-b* sequences found a single best tree of score  $-\ln L = 517.99024$ , and confirmed the existence of two main clades consisting of (1) birds from Australia and Otago and (2) birds from the rest of New Zealand. The analysis placed the Western Australian specimens in the Australian clade, clearly distinct from the New Zealand specimens. Within the Australian clade, however, there was no clear separation of Victorian and Western Australian specimens (Fig. 2).

The ML analysis of the control region sequences found a single best tree of score  $-\ln L = 999.9811$ . The phylogenetic analysis of the control region mitochondrial sequences also found the same two major clades as found from *cyt-b* and also placed the Western Australian blue penguins in a clade with the other Australian blue penguins (Fig. 3).

## DISCUSSION

Blue penguins from the east and west coasts of Australia showed few genetic differences in the portions of the *cyt-b* gene and the control regions we sequenced. Additionally, there was limited or shallow phylogeographic structure within the Australian clade, indicating that the populations on the two coasts of Australia plus the Otago population in New Zealand have occupied ranges free of impediments to gene flow (Avice 1994). Klomp & Wooller (1988) suggested that Western Australian blue penguins may

be larger because of a more favourable climate and food supply, as an alternative to genetic isolation. We agree that favourable food and/or climate are more likely than genetic isolation to have produced the morphological differences between populations breeding on the two coasts. Analysis of highly variable microsatellite regions of penguins breeding on both coasts of Australia and in Otago, which can uncover the population history of individual colonies and identify migration between colonies (Sunnucks 2000), would be a useful future project.

The taxonomy of *Eudyptula* in New Zealand has been somewhat controversial over the last 30 years. Kinsky & Falla (1976) reclassified the two *Eudyptula* species (*E. minor* and the white-flipped penguin *E. albosignata*) into a single species with six subspecies based on morphometric data such as bill size, body weight and tarsus length. Our results did not agree: Banks Peninsula birds were not genetically distinctive, and in any case, there are breeding birds with a white flipper margin in Oamaru (Hocken 1997). After Meredith & Sin (1988a,b) used an analysis of allozyme data from four populations to demonstrate a very high degree of intra- and inter-population variability within the New Zealand clade of *Eudyptula*, all subspecies were deleted (Turbott 1990). We conclude that there are few impediments to gene flow within the New Zealand taxon.

Cladistic classification systems recognise only monophyletic clades (a clade that includes all the descendants of a common ancestor) (Page & Holmes 1998) and our results confirm that there are only two clades of blue penguins in Australasia. The addition of sequences for *cyt-b* from the two Western Australian individuals and for the control region from 33 new specimens from various locations supported the distinction between two distinct genetic clades found in our previous study of fewer specimens (Banks et al. 2002). At Oamaru, north Otago, the two clades coexist and it would be interesting to examine breeding pairs to see whether they are hybridising. It would also be useful to examine more individuals from some of the areas we have not sampled as extensively, to investigate whether the same two genetic clades coexist at other colonies.

The *cyt-b* sequences of the two blue penguin clades were more different from each other (4% different), than African penguins, *Spheniscus demersus*, are from Magellanic penguins, *S. magellanicus* (1% different); or African penguins are from Humboldt penguins, *S. humboldti* (2% different) (Baker et al. 2006). Many closely related vertebrate taxa currently recognised as full species diverge from their sister

taxa by distances of 2% or more in *cyt-b* (Johns & Avise 1998), suggesting that reclassifying *Eudyptula minor* as two species may be warranted. Before *Eudyptula* is reclassified, however, we advocate sequencing of nuclear gene regions to investigate whether nuclear gene flow reflects mitochondrial gene flow. Also, identifying morphological characters that reliably distinguish the two clades will be difficult, as the morphology of the two groups overlaps extensively.

Our results suggest that the current conservation status of blue penguin as of “Least concern” (IUCN 2006) is justified, as both clades appear to be reasonably numerous. However, the decline in blue penguin population size in the last few decades, correlated with human activities (Challies & Burleigh 2004), should not be ignored, and more intensive management of threats to these populations may be required if it continues.

#### ACKNOWLEDGMENTS

Thanks to Roz Jessop, Peter Dann and Belinda Cannel for providing samples from the Australian blue penguins and Tony Hocken for the Oamaru samples. This work was partly funded by Lincoln University and a Kelly Tarlton’s Antarctica scholarship.

#### REFERENCES

- Avise JC 1994. Molecular markers, natural history and evolution. New York, Chapman and Hall. 511 p.
- Baker AJ, Pereira SL, Haddrath OP, Edge K 2006. Multiple gene evidence for expansion of extant penguins out of Antarctica due to global cooling. *Proceedings of the Royal Society B* 273: 11–17.
- Banks JC, Mitchell AD, Waas JR, Paterson AM 2002. An unexpected pattern of molecular divergence within the blue penguin (*Eudyptula minor*) complex. *Notornis* 49: 29–38.
- Challies CN, Burleigh RR 2004. Abundance and breeding distribution of the white-flipped penguin (*Eudyptula minor albosignata*) on Banks Peninsula, New Zealand. *Notornis* 51: 1–6.
- Daugherty CH, Cree A, Hay JM, Thompson MB 1990. Neglected taxonomy and continuing extinctions of tuatara (*Sphenodon*). *Nature* 347(6289): 177–179.
- del Hoyo J, Elliot A, Sargatal J 1992. Handbook of the birds of the world. In: del Hoyo J, Elliot A, Sargatal J ed. Barcelona, Lynx Edicions. 696 p.
- Freeland JR, Boag PT 1999. Phylogenetics of Darwin’s finches: paraphyly in the tree finches, and two divergent lineages in the warbler finch. *The Auk* 116(3): 577–588.
- Hasegawa M, Kishino H, Yano T 1985. Dating of the human-ape splitting by the molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22: 160–174.
- Hocken AG 1997. Plumage and bill morphology variations in a population of the blue penguin (*Eudyptula minor*). *Notornis* 44: 259–263.
- IUCN 2006. IUCN red list of threatened species. (Date accessed 4 May 2006 from <http://www.iucnredlist.org>.)
- Johns GC, Avise JC 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. *Molecular Biology and Evolution* 15(11): 1481–1490.
- Kinsky FC, Falla RA 1976. A subspecific revision of the Australasian blue penguin (*Eudyptula minor*) in the New Zealand region. *National Museum of New Zealand Records* 1(7): 105–126.
- Klomp NI, Wooller RD 1988. The size of little penguins *Eudyptula minor* on Penguin Island, Western Australia. *Records of the Western Australian Museum* 14(2): 211–215.
- Lambert DM, Ritchie PM, Millar CD, Holland B, Drummond AJ, Baroni C 2002. Rates of evolution in ancient DNA from Adelie penguins. *Science* 295: 2270–2273.
- Marchant S, Higgins PJ 1990. Handbook of Australian, New Zealand and Antarctic birds. Oxford, Oxford University Press.
- McNeely JA 2002. The role of taxonomy in conserving biodiversity. *Journal for Nature Conservation* 10: 145–153.
- Meredith M, Sin F 1988a. Genetic variation of four populations of the little blue penguin, *Eudyptula minor*. *Heredity* 60: 69–76.
- Meredith M, Sin F 1988b. Morphometrical analysis of four populations of the little blue penguin, *Eudyptula minor*. *Journal of Natural History* 22: 801–809.
- Nunn GB, Cooper J, Jouventin P, Robertson CJR, Robertson GG 1996. Evolutionary relationships among extant albatrosses (Procellariiformes: Diomedidae) established from complete cytochrome-b gene sequences. *The Auk* 113(4): 784–801.
- Page RDM, Holmes E 1998. Molecular evolution. A phylogenetic approach. Oxford, Blackwell Science. 346 p.
- Posada D, Crandall KA 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

- Sunnucks P 2000. Efficient genetic markers for population biology. *Trends in Ecology and Evolution* 15: 199–203.
- Turbott EG 1990. Checklist of the birds of New Zealand. 3rd ed. Auckland, Random Century. 247 p.
- Wienecke BC 1993. The size and breeding patterns of little penguins *Eudyptula minor* in Australia: a comparative study. Unpublished PhD thesis, Murdoch University, Perth.
- Yang Z 1993. Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution* 10(6): 1396–1401.