Evidence of an Australasian divide :)

exploring little penguins using DNA barcodes

John Waugh

The Debate

- The little penguin (*Eudyptula minor*) has been the subject of ongoing debate as to its precise species and/or subspecies delineation

- Up to six subspecies have been identified but at present the Checklist of the Birds of New Zealand recognises only one species and no subspecies

Table 1 The six subspecies that have been recognised in *Eudyptula minor*.[13] The current Checklist of the Birds of New Zealand[16] does not recognise any subspecies.

Subspecies	Distribution
Eudyptula minor novaehollandiae	Southern Australia and Tasmania
Eudyptula minor iredalei	Northern North Island (NZ)
Eudyptula minor variabilis	Southern North Island and Cook Strait (NZ)
Eudyptula minor albosignata	Eastern South Island (NZ)
Eudyptula minor minor	Western South Island and Stewart Island (NZ)
Eudyptula minor chathamenssis	Chatham Islands

The Debate

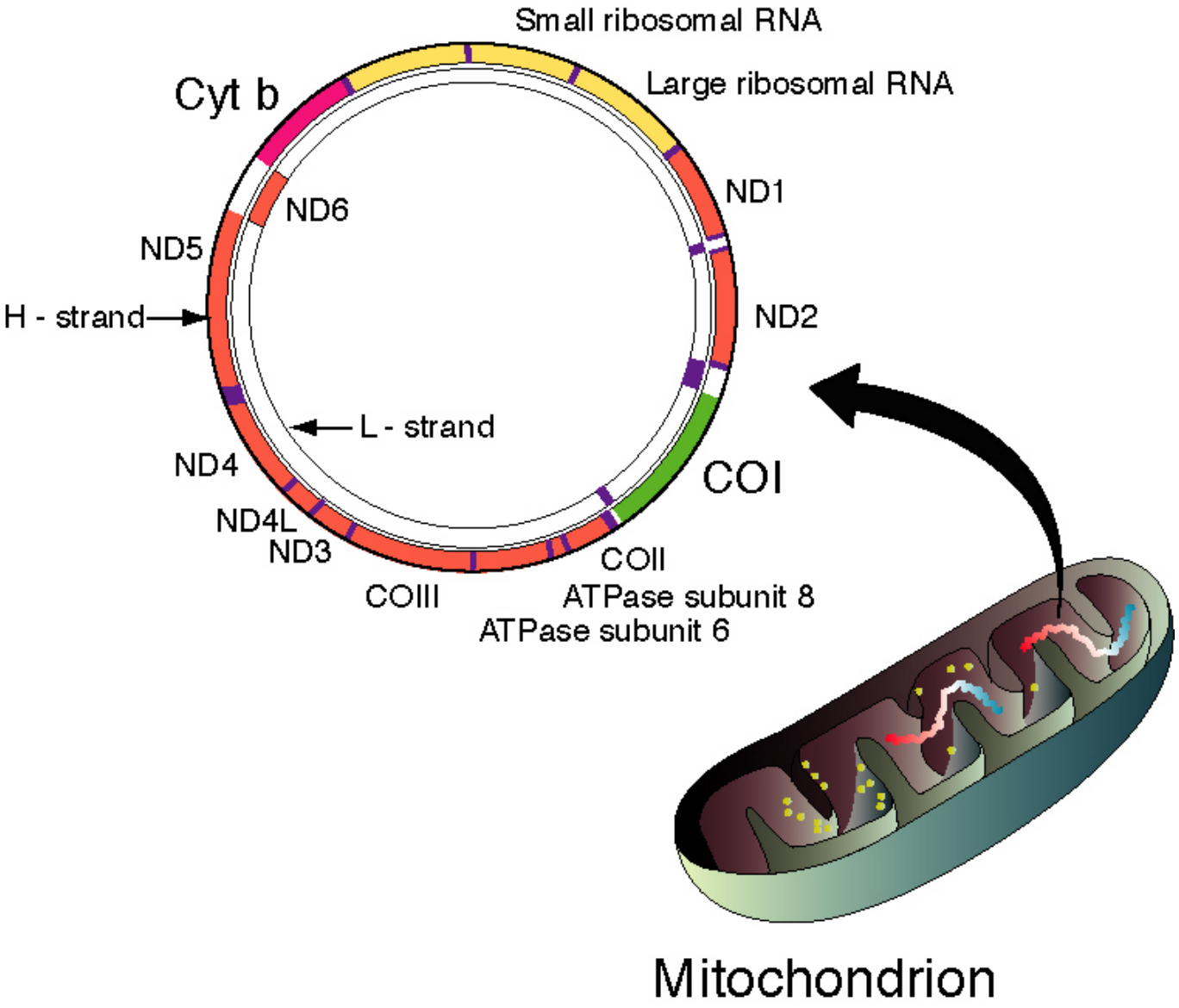
- More data is required to resolve this debate and DNA barcoding offers an additional source of objective data to contribute to this discussion

What are DNA barcodes?

- Haplotypic variation in a 648 bp segment of the cytochrome *c* oxidase subunit I (COI) gene within the mitochondrial genome, starting from base 58 at the 5’ end of the gene

- Intraspecific variation in this gene is generally <10% of that observed between congeneric species

Mitochondrial DNA



DNA barcoding

- Early in its use, it became apparent that it might highlight potential cryptic species and inform the debate around their status

Method

- Sequences from 57 specimens of *E. minor* were analysed using MrBayes software to generate Bayesian inference of phylogeny. One million cycles were run before a phylogenetic tree with the highest possible posterior probability was produced

- In addition, Kimura-2-parameter (K2P) genetic distance correction analysis was carried out

- Both these methods build phylogenetic trees based on genetic distance

Results

- Both the Bayesian tree and the K2P tree showed two distinct clades correlating to broad geographical location (Table 2 and Figs. 1 and 2)

Table 2 Kimura-2-Parameter genetic distance at the DNA barcode locus for 57 specimens of *Eudyptula minor* from Australia (n=23) and New Zealand (n=34).

	n	Mean Distance (%)	Max Distance (%)	se
Within species	57	1.874	4.21	0.046
Within NZ specimens	34	0.097	0.323	0.004
Within Aus specimens	23	0.055	0.292	0.005

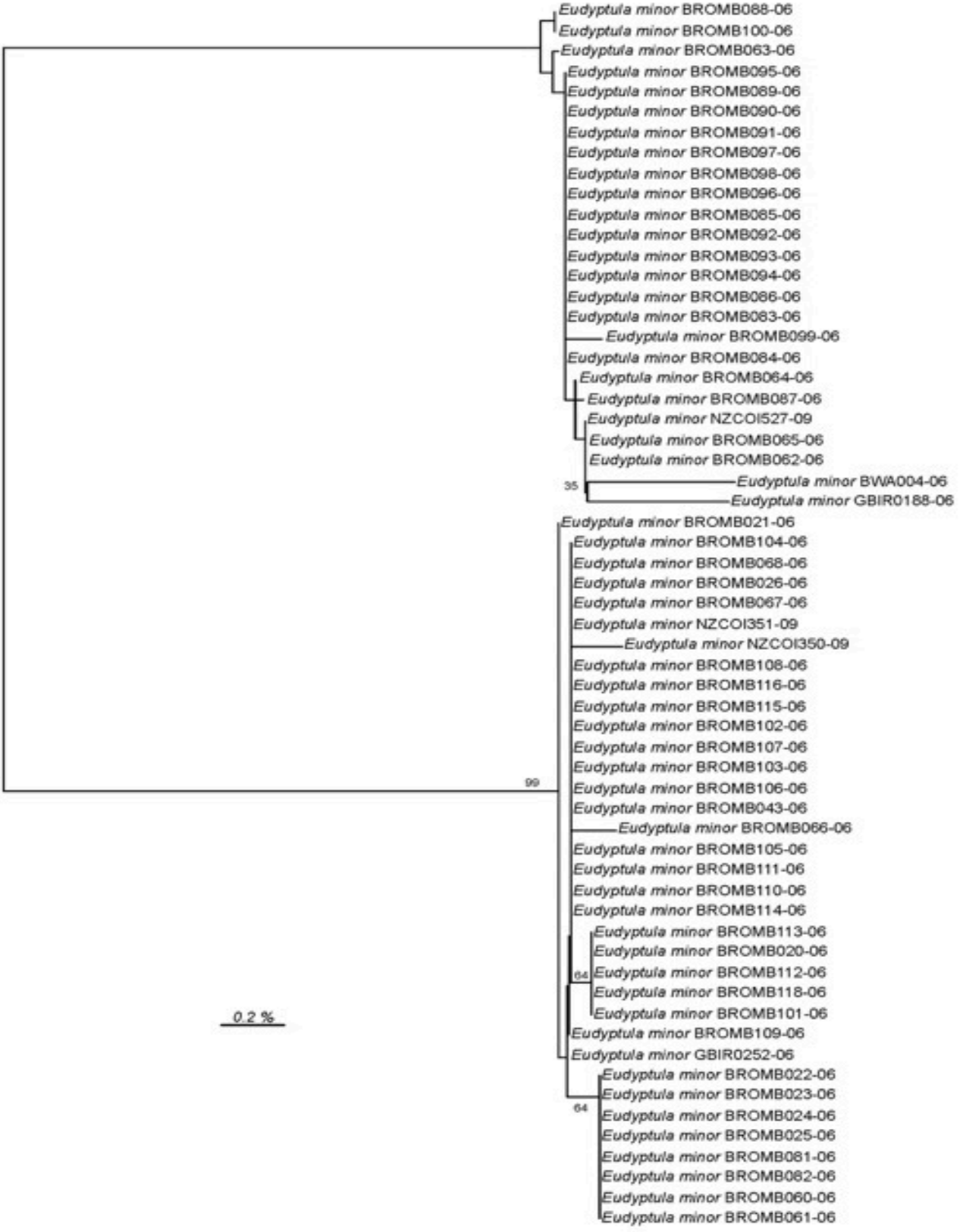


Figure 1 A Bayesian tree of the barcode region of the mitochondrial CO1 gene from specimens of *Eudyptula minor* collected in Western Australia, Victoria and various locations in the North and South Islands of New Zealand with bootstrap values for all major branches.

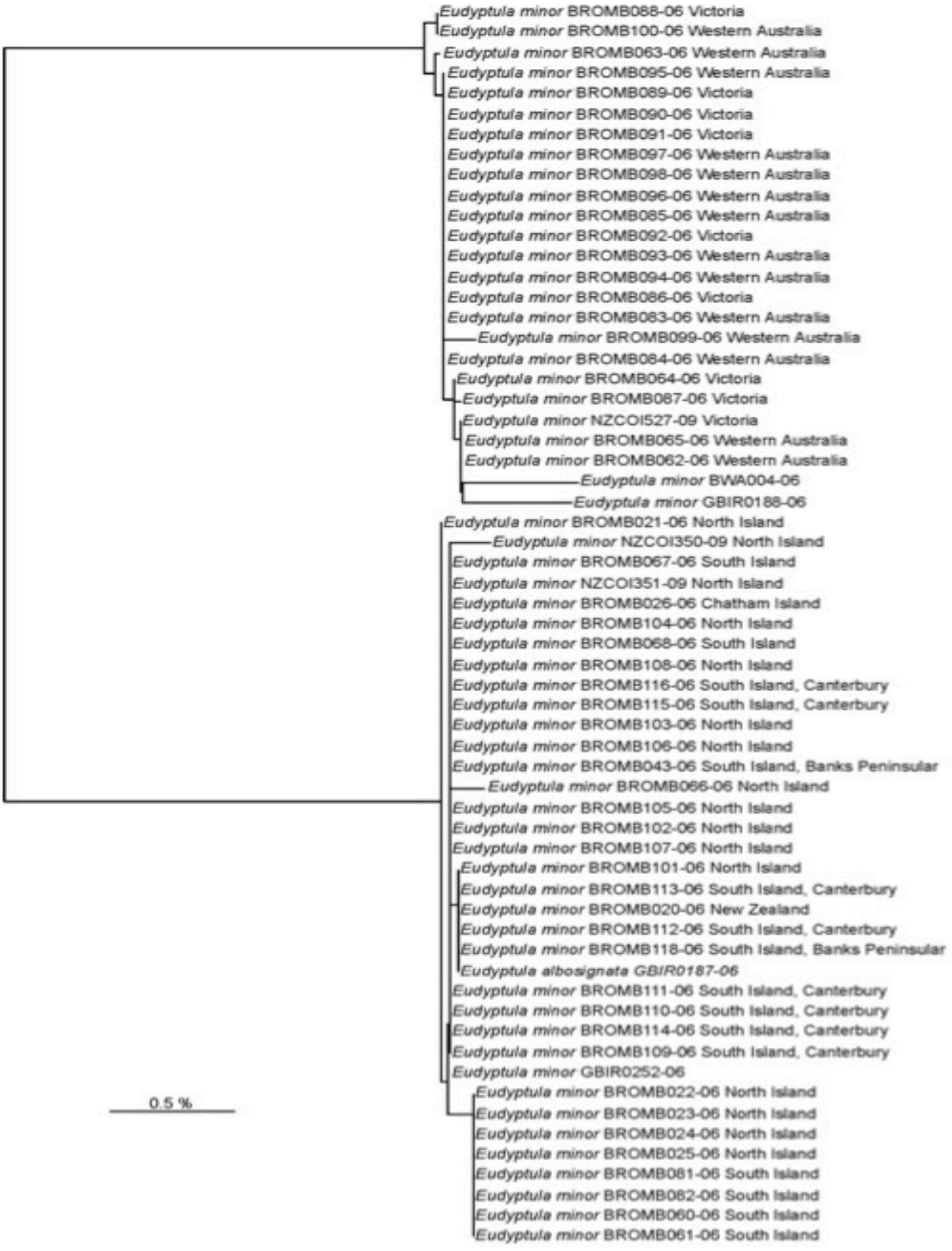


Figure 2 A Kimura-2-Parameter genetic distance tree of the barcode region of the mitochondrial CO1 gene from specimens of *Eudyptula minor* collected in Western Australia, Victoria and various locations in the North and South Islands of New Zealand.

Results

- 23 sites (nucleotide bases) within the barcode region differed between birds from the two geographic locations but did not differ between birds from the same broad geographic location

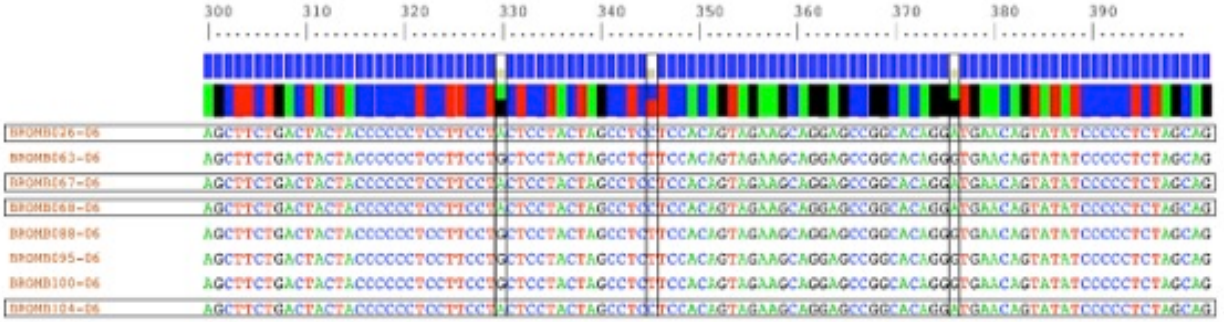


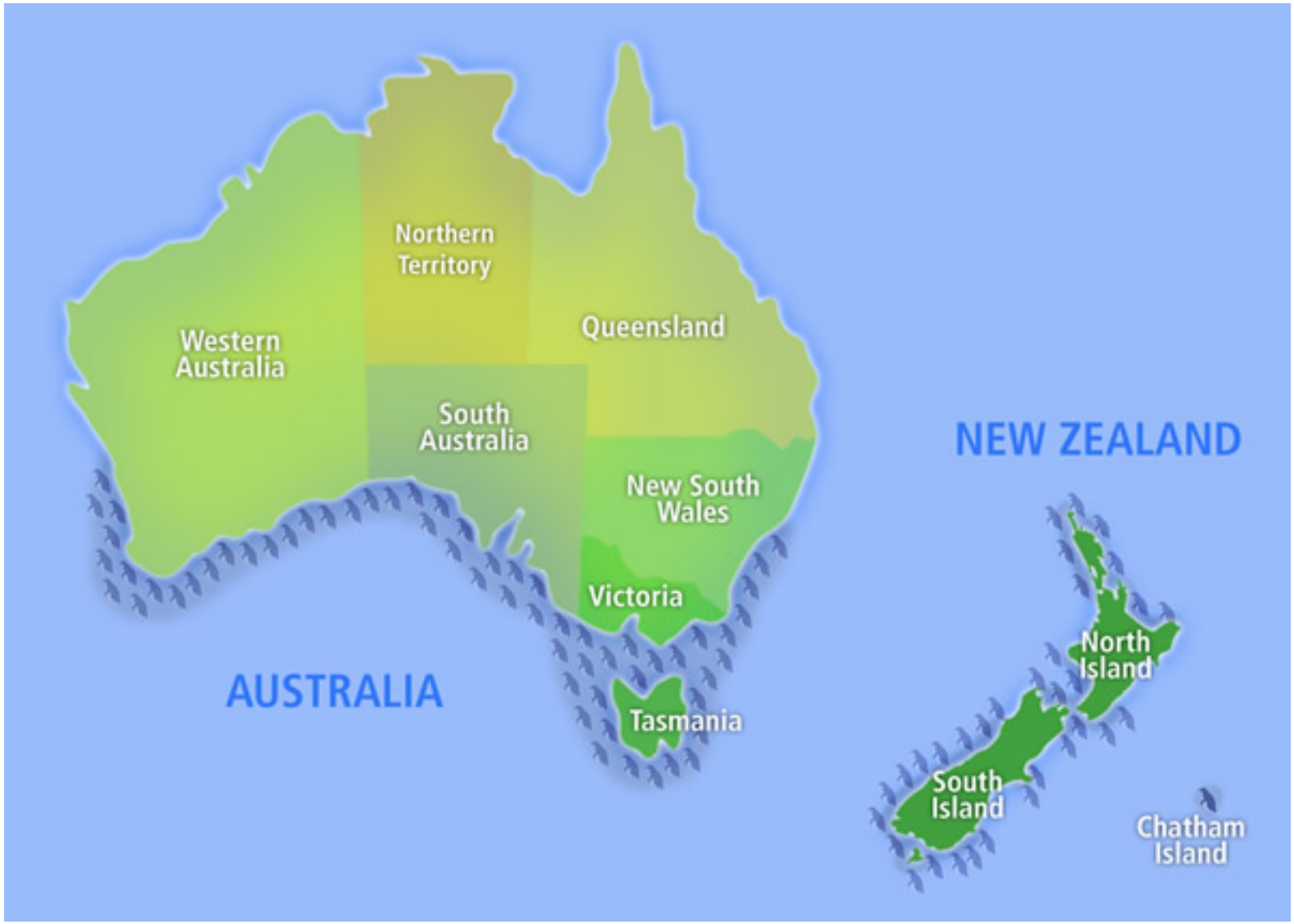
Figure 3 Part of an alignment, made using ClustalX version 0.1 (copyright 2003 Ramu & Co.), of sequences of the DNA barcode region *Eudyptula minor* specimens from New Zealand (framed with black lines) and Australia (unframed) showing 3 of the 23 sites at which specimens from these two regions differed.

Geographical isolation

- *E. minor* tends to remain within 25km of the shore during its foraging expeditions, precluding trans-Tasman excursions

- Australian specimens, although collected from widely separated regions, showed homogeneity at the barcode region. However, their distribution is along a continuous coastline or in the case of the Bass Strait, has numerous stepping stone islands

- The same is largely true for New Zealand specimens


Northern Territory
Western Australia
Queensland
South Australia
New South Wales
Victoria
AUSTRALIA
Tasmania
NEW ZEALAND
North Island
South Island
Chatham Island

Conclusion

- DNA barcodes provide evidence that Australian and New Zealand members of this genus form two distinct clusters

- COI divergence may not be sufficient evidence on its own to justify an investigation of species status. However, when correlated with the geographic separation of the populations involved as well as behavioural evidence, a compelling argument is made

Conclusion

- Where debate arises over the status of a species, DNA barcoding offers objective biological evidence that can augment existing taxonomic data

- In the case of *E. minor*, the DNA barcode data collected in this study support a two species theory

Acknowledgement

My thanks to the late Professor Allan Baker, who generously shared his data with me; a generosity that appeared to be a habit of life