Molecular Evidence for the Identity of the Magenta Petrel

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Running title: Molecular Identity of the Magenta Petrel

Abstract

A lone petrel was shot from the decks of an Italian warship (the '*Magenta*') while it was sailing the South Pacific Ocean in 1867, far from land. The species, unknown to science, was named the '*Magenta Petrel*' (Procellariiformes, Procellariidae, *Pterodroma magentae*). No other specimens of this bird were collected and the species it represented remained a complete enigma for over 100 years. We compared DNA sequence of the mitochondrial cytochrome *b* gene from the *Magenta Petrel* to that of other petrels using phylogenetic methods and ancient DNA techniques. Our results strongly suggest that the *Magenta Petrel* specimen is a Chatham Island Taiko. Furthermore, given the collection location of the *Magenta Petrel*, our finding indicates that the Chatham Island Taiko forages far into the Pacific Ocean (near South America). This has implications for the conservation of the Taiko, one of the world's rarest seabirds.

1 **Introduction**

2
3 A lone petrel was shot from the decks of the Italian warship *Magenta* while it was sailing the
4 central South Pacific Ocean in 1867 (Figure 1; Giglioli & Salvadori 1869). Similar individuals
5 were seen weeks later southeast of Rapa Nui / Easter Island and again north of the Juan
6 Fernandez Islands near Chile (Giglioli & Salvadori 1869). The species was unknown to science
7 and named the ‘Magenta Petrel’ (Procellariiformes, Procellariidae, *Pterodroma magentae*). The
8 single specimen was stored in the Turin museum and despite Second World War bombing the
9 Magenta Petrel survived (Crockett 1994). The Magenta Petrel became an enigma being never
10 collected subsequently, and never sighted again.

11 The Magenta Petrel type specimen is morphologically very similar to a number of other petrel
12 species and its identity has been the subject of conjecture. Over the intervening 120 years there
13 have been a number of suggestions regarding the identity of the Magenta Petrel. For example,
14 Fullagar and van Tets proposed the Magenta Petrel was a Phoenix Petrel (*P. alba*; Harrison
15 1983), while Bourne suggested it belonged to the extinct Chatham Island Taiko (*P. magentae*;
16 Bourne 1964). Amazingly, in 1978 the Taiko was rediscovered by David Crockett who captured
17 two individuals (Crockett 1994). Subsequently, some taxonomists considered the Magenta Petrel
18 and Chatham Island Taiko to be the same species (e.g. Marchant & Higgins 1990).

19 In an attempt to clarify the taxonomic status of the Magenta Petrel, we compared DNA
20 sequences from the type specimen to homologous sequences from 18 other petrel species and
21 from modern and ancient Chatham Island Taiko. We incorporated all species that are
22 morphologically similar to the Magenta Petrel (including the non-congeneric Tahiti Petrel,
23 *Pseudobulweria rostrata*; Marchant & Higgins 1990), and those that have been described in the

1 same subgenus. The subgenus '*Pterodroma*' includes Chatham Island Taiko, Murphy's Petrel
2 (*Pterodroma ultima*), Providence Petrel (*P. solandri*), Great-winged Petrel (*P. macroptera*),
3 White-headed Petrel (*P. lessonii*), Soft-plumaged Petrel (*P. mollis*), Zino's Petrel (*P. madeira*),
4 Fea's Petrel (*P. feae*), Atlantic Petrel (*P. incerta*), Black-capped Petrel (*P. hasitata*), and Cahow
5 (*P. cahow*; Imber 1985).

8 **Materials and Methods**

10 *DNA Samples and Extraction*

12 A blood sample of the Grey-faced Petrel (*Pterodroma macroptera gouldi*) was collected and
13 stored in Queen's lysis buffer (Seutin *et al.* 1991). DNA was extracted by proteinase K digestion
14 and a modified version of the phenol / chloroform method (Sambrook *et al.* 1989, Lawrence *et*
15 *al.* 2008a).

16 Feather and tissue samples were obtained from the Magenta Petrel type specimen that is stored
17 at the Museo Regionale di Scienze Naturali Torino, Italy. Feather samples were obtained from
18 Phoenix Petrel (Av3886), White-necked Petrel (*P. cervicalis*, Av1313), Juan Fernandez Petrel (*P.*
19 *externa*, Av1305) and Murphy's Petrel (Av1320) housed at the Canterbury Museum,
20 Christchurch.

21 DNA extraction of feather and tissue samples and polymerase chain reaction (PCR) set up was
22 performed in a dedicated, physically isolated ancient DNA laboratory. Access of personnel to the
23 laboratory was controlled with only 'one-way traffic' of people, samples, reagents, and

equipment. That is, movement from ancient to modern laboratory; never the reverse. Surfaces, pipettes and other implements were cleaned with 10% sodium hypochlorite then 80% ethanol before use. DNA extraction and pre-PCR set up were conducted in separate rooms. The bench used for PCR set up was UV-irradiated each night. Contamination was monitored using extraction and PCR negative controls. DNA was isolated by proteinase K / DTT / SDS digestion and phenol / chloroform extraction (Sambrook *et al.* 1989), then using a QIAamp[®] DNA Mini Kit (Qiagen) following the post-lysis tissue extraction protocol.

DNA Amplification and Sequencing

The entire mitochondrial cytochrome *b* gene was amplified and sequenced from DNA extracted from the Grey-faced Petrel blood sample as in Lawrence *et al.* (2008a). For all other samples, we amplified three regions of the cytochrome *b* gene (totalling 303bp) by PCR (details of these regions in Lawrence *et al.* 2008b). Primers included: L14863 (Nunn *et al.* 1996) and HCytB21-55 (Lawrence *et al.* 2008b); LCytB432 and HCytB571 (Lawrence *et al.* 2008a); and LCytB679 and HCytB780 (Lawrence *et al.* 2008b). PCR reaction mixtures contained 2-4µl of DNA extract, 1X PCR buffer (Invitrogen[™]), 1.5mM – 3mM MgCl₂, 200µM of each dNTP, 2mg/ml BSA, 0.2µM of each primer, 1-2 units of Platinum[®] taq polymerase, and distilled water to a final volume of 20µl. For the third region, the Magenta Petrel sample was amplified by PCR containing 50mM Tris-HCl (pH8.8) and 20mM (NH₄)₂SO₄ instead of Invitrogen[™] buffer. The PCR amplification profile was: hot start 94°C for 2 min, 10 cycles of 94°C for 20 seconds (sec), 52°C for 30 sec, 72°C for 30 sec; then 50 cycles of 94°C for 20 sec, 50°C for 30 sec, 72°C for 30

1 sec; then a final extension at 72°C for 10 mins. PCR products were purified using a QIAquick®
2 PCR purification kit (Qiagen) then sequenced on an Applied Biosystems 3730 DNA analyser by
3 the AWC Genome Service (Auckland, New Zealand). The procedure was independently
4 replicated and the sequences verified for Magenta Petrel samples at a dedicated ancient DNA
5 facility at the University of Auckland. The three regions of cytochrome *b* were concatenated and
6 sequences were codon aligned using Sequencher™ version 4.2.2 (GeneCodes). Sequences were
7 deposited in the NCBI GenBank®; accession numbers EU979352 – EU979357. Sequences from
8 other petrel species (and a shearwater) were obtained from Genbank® (accession numbers:
9 U74331-U74337, U74341, U74346-U74347, U74353, U74655). Note the sequence of Fea's
10 Petrel (*Pterodroma feae*) is named in Genbank® as *P. deserta* (Penhallurick & Wink 2004).
11 Cytochrome *b* sequence from a light-morph Trindade Petrel (*P. arminjoniana*) was kindly
12 provided by Ruth M. Brown (unpubl. data). Sequences were obtained from modern and ancient
13 Chatham Island Taiko (Lawrence 2008a; Lawrence 2008b). Only partial cytochrome *b* sequence
14 was available from Tahiti Petrel (496bp, beginning 99bp from the 5' end) and Zino's Petrel
15 (473bp, beginning 345bp from 5' end, Genbank® accession numbers: U70482 and U74653
16 respectively). All species (except Taiko) were represented by single sequences in analyses.

18 *Phylogenetic Analyses*

20 We estimated phylogenies by Bayesian inference with MrBayes 3.1.2 (Ronquist & Huelsenbeck
21 2003), and maximum likelihood and maximum parsimony analyses using Paup* 4.0b10
22 (Swofford 2002). Models of sequence evolution were selected using Modeltest 3.7 (Posada &

Crandall 1998). The first set of trees was constructed using the HKY+I model with 150bp of sequence from all species to determine the position of Tahiti Petrel and Zino's Petrel (from which only short sequence was available). For Bayesian analysis four chains were run for 6 million generations (temperature = 0.2), trees were sampled every 100 generations. Following a 20% burn-in a 50% majority rule consensus tree was constructed. A maximum likelihood tree was constructed with 1000 bootstrap replicates, using a heuristic search with random sequence replicates and TBR branch swapping. Strict and 50% majority rule consensus maximum parsimony trees were constructed using a heuristic search with random sequence replicates. Tahiti Petrel diverged close to the outgroup and Zino's Petrel in a completely different clade to the Magenta Petrel, with a Bayesian probability of 99%, and maximum likelihood bootstrap value of 74. There were two nucleotide differences between Zino's Petrel and its closest relative Fea's Petrel, and six nucleotide differences between Zino's Petrel and Taiko. Thereafter, the same method was used for 303bp of cytochrome *b* sequence from all other petrels that are morphologically similar to the Magenta Petrel and those that have been described in the same subgenus. Trees were rooted with the outgroup Sooty Shearwater (*Puffinus griseus*).

Results

The Magenta Petrel sequence was identical to the most common haplotype recorded from both modern (8 haplotypes $N=90$; Lawrence *et al.* 2008a) and ancient Taiko (8 haplotypes $N=44$; Lawrence *et al.* 2008b; Figure 1). Phylogenetic analyses of DNA sequences recovered from the Magenta Petrel type specimen compared to other petrels indicated the Magenta Petrel and the

Chatham Island Taiko are indistinguishable genetically (Figure 1). The Magenta Petrel grouped as a monophyletic clade with Taiko in all trees (Bayesian inference, maximum likelihood and maximum parsimony) with strong posterior probability and bootstrap support (Figure 1). The number of nucleotide substitutions between Taiko haplotypes ranged from 1-2bp. Nucleotide substitutions between the Magenta Petrel and other *Pterodroma* species (except Taiko) ranged from 9-30bp. There were no indels or amino acid substitutions between the Magenta Petrel and the other species, indicating the sequences were homologous.

Discussion

Our results show that for the mitochondrial cytochrome *b* region we DNA sequenced, the Magenta Petrel has the most common haplotype found in the Chatham Island Taiko. This finding could be interpreted as shared ancestry of a common haplotype by the Taiko and a closely related petrel species, the Magenta Petrel being a hybrid between Taiko and another petrel species, or as a result of DNA contamination. However, given the precautions taken to avoid contamination and the morphological similarities between the Magenta Petrel and Taiko our results strongly suggest that the Magenta Petrel and the Chatham Island Taiko are the same species (Figure 1). The only known population of the Taiko is critically endangered and is located on Chatham Island / Rekohu / Wharekauri (Onley & Scofield 2007). The population is estimated to number between 120-150 birds including just 8-15 breeding pairs. Little is known about the habits of the Taiko outside of the breeding season (Onley & Scofield 2007). The Magenta Petrel was collected in the central South Pacific Ocean and seen southeast of Rapa Nui / Easter Island and near the

1 Juan Fernandez Islands, far from the Chatham Islands, 140 years ago (Giglioli & Salvadori 1869;
2 Figure 1). Interestingly, there have also been more recent reported sightings of the Taiko at sea
3 off the coast of central Chile (Howell *et al.* 1996; Figure 1). This suggests that the Taiko forages
4 far into the South Pacific. This has implications for the conservation of the Taiko, one of the
5 world's rarest seabirds.

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Fig. 1 The relationship among cytochrome *b* sequences from the Magenta Petrel (*Pterodroma magentae*), morphologically similar and closely related petrels. The approximate location of the collection site of the type specimen of the ‘Magenta Petrel’ is indicated by X within a red circle, other reported sightings are indicated by orange circles. The dashed line represents the voyage of the *Magenta* (Giglioli 1875). Below, a Bayesian inference consensus tree shows the phylogenetic relationship of the Magenta Petrel type specimen to a range of *Pterodroma* petrels (¹ Juan Fernandez Petrel *Pterodroma externa*, ² White-necked Petrel *P. cervicalis*, ³ Murphy’s Petrel *P. ultima*, ⁴ Phoenix Petrel *P. alba*, ⁵ Kermadec Petrel *P. neglecta*, ⁶ Trindade Petrel *P. arminjoniana*, ⁷ Mottled Petrel *P. inexpectata*, ⁸ Providence Petrel *P. solandri*, ⁹ Cahow *P. cahow*, ¹⁰ Fea’s Petrel *P. feae*, ¹¹ Black-capped Petrel *P. hasitata*, ¹² Grey-faced Petrel *P. macroptera gouldi*, ¹³ Great-winged Petrel *P. macroptera macroptera*, ¹⁴ White-headed Petrel *P. lessonii*, ¹⁵ Atlantic Petrel *P. incerta*, ¹⁶ Soft-plumaged Petrel *P. mollis*), constructed using partial cytochrome *b* sequences with Sooty Shearwater, *Puffinus grisues* as an outgroup (O). Bayesian inference posterior probabilities of clades have been converted to the percentage of trees with that topology and are presented to the left of the branch. Bayesian inference, maximum likelihood, and maximum parsimony trees all concurred except where a grey circle indicates a node that occurred in both Bayesian inference and maximum likelihood trees, but not maximum parsimony. Pie charts show haplotype frequencies in ancient (*N*=44) and modern Taiko (*N*=90). The haplotype of the Magenta Petrel is represented in red.