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Using ancient DNA to quantify losses of genetic and species diversity in seabirds: a case study of *Pterodroma* petrels from a Pacific island

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Abstract

The largest anthropogenic extinction events during the Holocene occurred on Pacific islands, where thousands of bird populations were lost. Although ancient DNA approaches have become widely used to monitor the genetic variability of species through time, few studies have been conducted to identify the potential cryptic loss of genetic and species diversity within Pacific seabird species. Here we used heterochronous sampling of mitochondrial DNA (Cytochrome *b*) in the genus *Pterodroma* from Norfolk Island to quantify potential loss of genetic and species diversity. We particularly focused on the providence petrel *P. solandri* whose main breeding colony (~ 1,000,000 breeding pairs) became extirpated from Norfolk Island following European settlement circa 1800. We sampled subfossil bones consistent with *Pterodroma* spp. from Norfolk Island, and performed genetic comparisons with other populations of *P. solandri* and congeneric species. The majority of subfossil Norfolk Island individuals exhibited the most common mitochondrial haplotype from Lord Howe Island *P. solandri*, suggesting no appreciable loss of genetic variation as a consequence of the Norfolk Island extirpation. Our findings provide an example where a large seabird population was rapidly extirpated by humans without loss of species-level genetic diversity, probably as a consequence of high connectivity with other populations. However, past connectivity was insufficient to prevent the extirpation itself, which has conservation implications for predicting the resilience of threatened seabirds. In contrast, ancient DNA analyses of smaller *Pterodroma* bones from Norfolk Island indicate the loss of a second species, potentially *P. pycrofti*, *P. brevipes* or another closely related, possibly undescribed taxon, from the Tasman Sea.

Keywords Ancient DNA · High-throughput DNA sequencing · Seabird · Petrels · Population genetics · Phylogenetics · Phylogeography · *Pterodroma* · Tasman sea

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Introduction

Global biodiversity loss due to human-induced environmental changes during the Holocene is accelerating at such an exceptional rate that biodiversity change is now considered an important global change in its own right (Walker 1996; Sala et al. 2000). In particular, the fauna and flora of Pacific islands are well known for their high extinction rates following human colonisation in the last millennium (Milberg and Tyrberg 1993; Smith et al. 1993; Steadman 2006, 1989). The impacts on birds were severe, with widespread and catastrophic extirpations in the order of 8000 island populations (Holdaway 1989; Pimm et al. 1994; Steadman 1995). However, the magnitude of overall genetic and species diversity loss accompanying these island extirpations is difficult to quantify in many instances because of limitations on specimen availability and the characters that can be analysed from them. Lineages with strong population genetic structure may have experienced massive loss of genetic diversity, including potentially cryptic species (Bálint et al. 2011; Niemiller et al. 2013). However, over-estimation of biodiversity loss is also possible in cases where apparently extinct taxa are subsequently shown to be conspecific with extant species (Steeves et al. 2010). Therefore, studies should document population-level variation to accurately quantify changes in genetic and species diversity (Beheregaray and Caccone 2007; Bickford et al. 2007; Brodersen and Seehausen 2014; Schwartz et al. 2007) that followed human colonisation of Pacific islands. Accurate quantification of these losses provides an important framework to predict and manage future threats to biodiversity in this region.

Ancient DNA methods to quantify losses of genetic and species diversity

Ancient DNA (aDNA) analyses have revolutionised the field of conservation genetics, as specific conservation issues may be informed by genetic studies of historical populations (Hofreiter et al. 2001; Leonard 2008; Orlando and Cooper 2014). Most commonly this involves reconstructing demographic histories and testing hypotheses about the timing of population changes (e.g. coincidence of decline and human colonisation; Brüniche-Olsen et al. 2018; Wilmshurst et al. 2014). Alternatively, aDNA studies may document cryptic losses of genetic and species diversity (e.g. Boessenkool et al. 2009; Calvignac et al. 2008; Ramírez et al. 2013), even describing previously unrecognized extinct lineages (Paxinos et al. 2002). For instance, using mitochondrial aDNA, Calvignac et al. (2008) documented declines in genetic diversity of the brown bear, *Ursus arctos*. In seabirds, although few studies to date have used heterochronous data sets for conservation purposes, Eda et al. (2011) combined classical paleontological data with ancient and modern DNA data to infer the ancient population structure of the short-tailed albatross *Phoebastria albatrus* and showed that the birds from the current two breeding regions (Torishima in the Izu Islands and two islets of the Senkaku Islands) descended from two ancient populations between which the genetic distance was greater than that of distinct sister albatross species, which implied the need of a re-evaluation of the status for the species. However, the potential of ancient DNA studies for quantifying genetic and species diversity changes of seabirds on Pacific islands following human colonisation is presently under-exploited, despite a wealth of Holocene subfossil material (Holdaway and Worthy 1994; Worthy and Holdaway 1993).

Pterodroma species from Norfolk Island

Norfolk Island (34.6 km^2) is located 600 km off the east coast of Australia (Fig. 1) and is one of a group of so-called 'Mystery Islands' in the southwest Pacific, having been deserted by Polynesians prior to discovery and colonisation by Europeans (Kirch 1988). The present-day avifauna on Norfolk Island is relictual following the extirpation of taxa such as *Pterodroma pycrofti* after Polynesian settlement (Holdaway and Anderson 2001), and *P. solandri* following European settlement in the late eighteenth century (Medway 2002a). The latter extirpation was dramatic and well documented, with around 1,000,000 pairs previously breeding on the island (Medway 2002a). The main contributors to population decline were direct exploitation by humans to avoid famine ($\sim$ 1,000,000 adults and young harvested in the four breeding seasons from 1790 to 1793 alone; Medway 2002a), along with predation and disruption of breeding by introduced mammals (pigs, rats; Schodde et al. 1983).

In addition to *P. pycrofti* and *P. solandri,* three other species of *Pterodroma* are known to breed on Norfolk Island: *P. cervicalis*, *P. nigripennis* and *P. neglecta* (Holdaway and Anderson 2001). These species may have bred on Norfolk Island prior to European arrival, and hence could provide insight into species attributes that enabled their survival during the associated perturbation. However, the small fragmentary bones common in middens are particularly difficult to identify (Holdaway and Anderson 2001). *Pterodroma solandri* bones are distinguishable from those of the other sympatric species of *Pterodroma* based on their size. Meredith (1985) recorded an unnamed smaller *Pterodroma* from subfossil

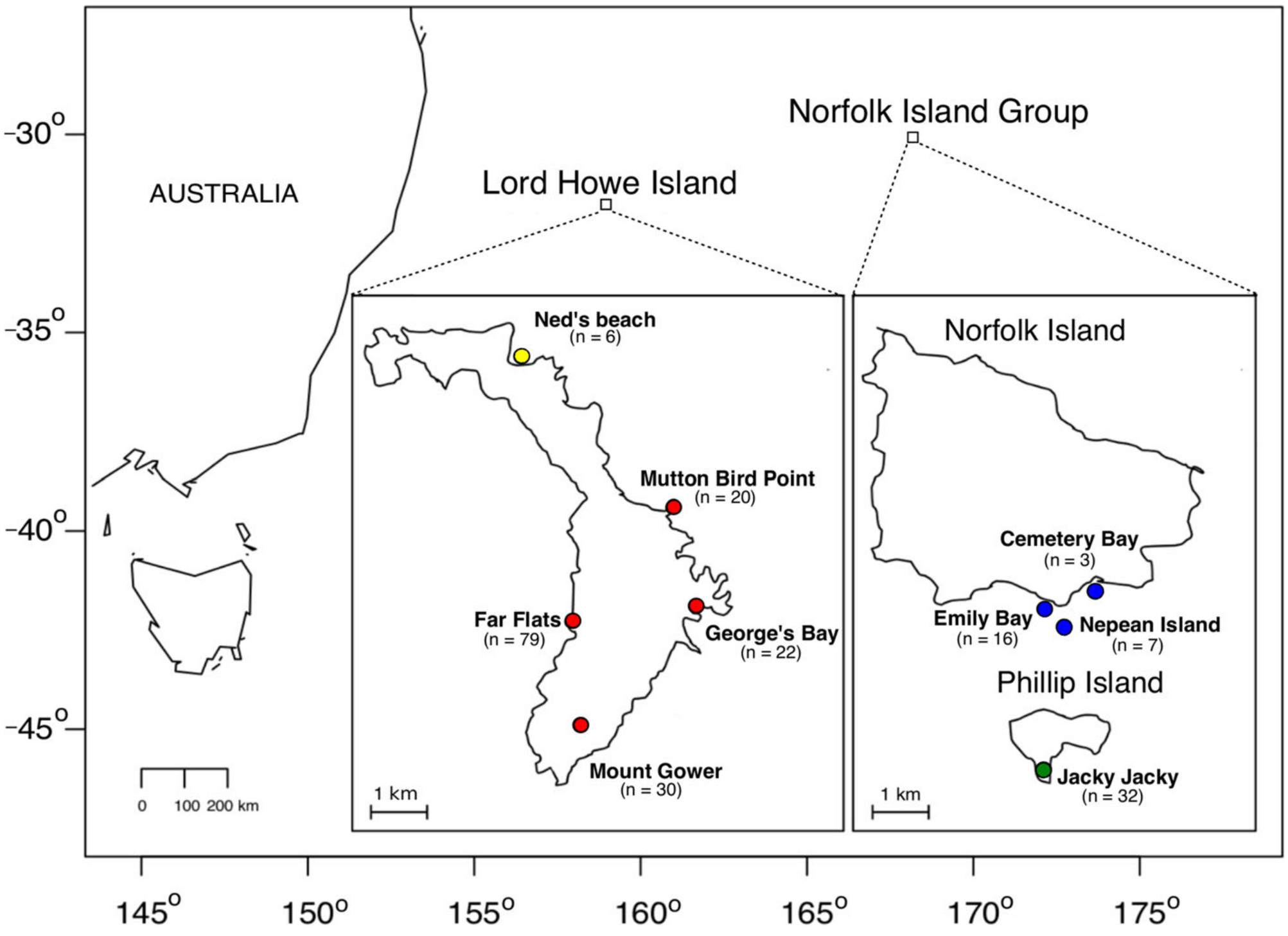

Fig. 1 Collection locations for *Pterodroma* samples. Red: Modern *P. solandri* samples collected on Lord Howe Island (n = 151); green: modern *P. solandri* samples collected on Phillip Island (n = 32). Total number of modern samples successfully sequenced = 176. Yellow: ancient *Pterodroma* samples collected on Lord Howe Island (n = 6); blue: ancient *Pterodroma* samples collected on Norfolk Island (n = 26)

deposits now thought to be *P. pycrofti* (see Holdaway and Anderson 2001). The dimensions and colour pattern of a small *Pterodroma* painted at the time of first European settlement (Hindwood 1965) were considered to fit those of *P. pycrofti* (Holdaway and Anderson 2001), although Whitley (1938) described the bird represented by the painting as a new species, *Cookilaria hindwoodi*, which is presently recognised as a synonym of *P. nigripennis*.

Although *P. solandri* was considered extirpated within the Norfolk Island group, a small population ($\sim$ 20 breeding pairs) was discovered on Phillip Island, 7 km south of Norfolk Island, in 1986 (Hermes et al. 1986) (Fig. 1). Genetic analysis revealed this population was recently founded by individuals from Lord Howe Island (Lombal et al. 2017), 900 km southwest of Norfolk Island (Fig. 1), representing the only substantial contemporary breeding locality of this species ($\sim$ 32,000 breeding pairs; Bester 2003). Despite suggestions of genetic connectivity based on the Phillip Island population, it is not known whether the Norfolk extirpation represented loss of genetic or even cryptic species diversity (see review Ramakrishnan and Hadly 2009). While there is no morphological evidence justifying taxonomic separation, the Norfolk Island population differed behaviourally, and such differences can constitute barriers to genetic exchange in seabirds (e.g. allochrony; Smith and Friesen 2007). Lord Howe Island individuals predominantly return to the colony during daylight (Bester et al. 2002; Medway 2002b), while nocturnal return was reported for the Norfolk Island population, and may relate to the presence of diurnal aerial predators (brown goshawks, *Accipiter fasciatus*) at the time of European settlement (Medway 2002b). However, Phillip Island individuals return only after dusk in the absence of these predators (A. Lombal and A. Tennyson pers. obs.), and hence this behavioural difference may be quite plastic, and not inhibit the homogenisation of genetic variation among populations.

In this study, we used ancient DNA methods to compare mitochondrial Cytochrome *b* DNA sequences from Norfolk Island Holocene subfossils to those of modern petrels. Our main goal was to test whether genetic diversity has been lost from Norfolk Island *Pterodroma* based on the analysis of subfossil material ascribed to *P. solandri* and other possible taxa.

Materials and methods

Sampling, ancient DNA extractions and high-throughput sequencing

Sixteen subfossil specimens were derived from excavations at Emily Bay on Norfolk Island between 1995 and 1997, that correspond to the 13th to 15th century Polynesian settlement of the island (Anderson and White 2001; Holdaway and Anderson 2001), and held at the Museum of New Zealand Te Papa Tongarewa (Table 1). Ten additional specimens were derived from four Holocene palaeontological excavations (Meredith 1985, 1991), held at the Australian National Wildlife Collections (ANWC; Table 1). Among these 26 specimens, 10 were provisionally morphologically identified as *P. solandri*, 10 were unable to be identified to species, and six were identified as *P. pycrofti* (Table 1). Although all *Pterodroma* humeri larger than those of *P. pycrofti* were of a size range consistent with specimens of *P. solandri*, different size classes in the leg elements were evident, suggesting that multiple taxa could be represented. We included all *Pterodroma* subfossil specimens (humeri) in our analyses. Additional subfossil specimens collected on

Table 1 Ancient samples obtained from bones at the Museum of New Zealand Te Papa Tongarewa (Te Papa) and the Australian National Wildlife Collections (ANWC) representing Lord Howe Island (LHI) and Norfolk Island (NI) specimens, including morphological identification

Accession #	ACAD #	Locality		Morphological ID	GenBank Accession #
Te Papa Museum					
S45698.1	20192	NI	Emily Bay*	*P. solandri*	MH828424
S45700.2	20193	NI	Emily Bay*	*P. solandri*	MH828425
S45702.3	20194	NI	Emily Bay*	*P. solandri*	MH828426
S45703.2	20195	NI	Emily Bay*	*P. solandri*	MH828427
S45704.5	20196	NI	Emily Bay*	*P. solandri*	MH828428
S45708	20197	NI	Emily Bay	*P. solandri*	
S45710.1	20198	NI	Emily Bay*	*P. solandri*	MH828429
S45710.2	20199	NI	Emily Bay	*P. solandri*	
S45710.3	20200	NI	Emily Bay*	*P. solandri*	MH828430
S45710.4	20201	NI	Emily Bay	*P. solandri*	
S45699.2	20202	NI	Emily Bay*	*P. pycrofti*	MH828431
S45701.1	20203	NI	Emily Bay*	*P. pycrofti*	MH828432
S45705.2	20204	NI	Emily Bay*	*P. pycrofti*	MH828433
S45706.2	20205	NI	Emily Bay	*P. pycrofti*	
S45707.2	20206	NI	Emily Bay*	*P. pycrofti*	MH828434
S45709.1	20207	NI	Emily Bay*	*P. pycrofti*	MH828435
Australian National Wildlife Collections (ANWC)					
ANWC P00126	17915	NI	Cemetery Bay*	*Pterodroma* sp.	MH828438
ANWC P00127	17916	NI	Cemetery Bay	*Pterodroma* sp.	
ANWC P00128	17917	NI	Cemetery Bay*	*Pterodroma* sp.	MH828439
ANWC P00132	17921	NI	Nepean Island	*Pterodroma* sp.	
ANWC P00133	17922	NI	Nepean Island*	*Pterodroma* sp.	MH828443
ANWC P00134	17923	NI	Nepean Island*	*Pterodroma* sp.	MH828444
ANWC P00135	17924	NI	Nepean Island	*Pterodroma* sp.	
ANWC P00136	17925	NI	Nepean Island	*Pterodroma* sp.	
ANWC P00137	17926	NI	Nepean Island*	*Pterodroma* sp.	MH828445
ANWC P00138	17927	NI	Nepean Island	*Pterodroma* sp.	
ANWC P00129	17918	LHI	Ned's Beach*	*P. solandri*	MH828440
ANWC P00130	17919	LHI	Ned's Beach*	*P. solandri*	MH828441
ANWC P00131	17920	LHI	Ned's Beach*	*P. solandri*	MH828442
ANWC P00123	17912	LHI	Ned's Beach	*P. solandri*	
ANWC P00124	17913	LHI	Ned's Beach*	*P. solandri*	MH828436
ANWC P00125	17914	LHI	Ned's Beach*	*P. solandri*	MH828437

*Successful ancient DNA extraction and sequencing

Lord Howe Island and identified as *P. solandri* were obtained from ANWC and analysed in the present study (n = 6, see details in Table 1).

DNA extraction and high-throughput sequencing was attempted on all ancient bone samples (n = 32). The Te Papa samples (n = 16) were processed in the dedicated ancient

DNA facilities at the Australian Centre for Ancient DNA (ACAD) at the University of Adelaide while the ANWC samples (n = 16) were processed in another clean-room at the University of Adelaide. Strict protocols were followed and a number of precautions taken to minimize contamination of samples with exogenous DNA (Cooper 2000). Potential surface contamination on the samples was reduced by UV irradiation for 15 min each side followed by abrading the exterior surface (c. 1 mm) using a Dremel tool and a disposable carborundum disk. The sample was then pulverized with a hammer and approximately 100 mg of powder used for extraction. DNA was extracted using an in-house silica-based extraction protocol adapted from Dabney et al. (2013). The powder was decalcified in 1 mL 0.5 M EDTA for 60 min, followed by an overnight digestion in 1 mL fresh 0.5 M EDTA containing 600 μg proteinase K at 55 °C. The samples were centrifuged and the supernatant mixed with 13 mL of a modified PB buffer (Qiagen) containing 0.0005% Tween-20 and 0.09 M sodium acetate and bound to silica dioxide particles, which were then washed twice with 80% ethanol. The DNA was eluted from silica particles with 100 μL TE buffer. Extraction blank (negative) controls were processed alongside the samples.

Double-stranded Illumina libraries were built following the protocol of Meyer and Kircher (2010) from 20 or 25 μL of DNA extract, for the ANWC and Te Papa samples, respectively, with the addition of truncated Illumina adapters with unique dual 7-mer internal barcodes to allow identification and exclusion of any downstream contamination. In addition, the Te Papa samples were subjected to the partial uracil-DNA glycosylase (UDG) treatment (Rohland et al. 2015). A short round of PCR was performed to increase the total amount of DNA using primers complementary to the adapter sequence (IS7 and IS8; Meyer and Kircher 2010). The PCR cycle number for the Te Papa samples was determined using real-time PCR (see Gamba et al. 2016) following the protocol described in Cascini et al. (2018), while the ANWC samples were arbitrarily subjected to 18 cycles of PCR. Each library was split into eight separate PCRs per library to minimize PCR bias and maintain library complexity. Each PCR of 25 μL contained 1 × HiFi buffer, 2.5 mM $MgSO_4$, 1 mM dNTPs, 0.5 μM each primer, 0.1 U Platinum Taq Hi-Fi polymerase and 2 μL DNA. The cycling conditions were 94 °C for 12 min, 13–23 cycles of 94 °C for 30 s, 60 °C for 30 s, and 68 °C for 40 s, followed by 68 °C for 10 min. PCR replicates were pooled and purified using AxyPrep (Axygen) magnetic beads for the Te Papa samples and AMPure (Agencourt) magnetic beads for the ANWC samples. DNA was eluted in 30 μL EB buffer and quantified with a Qubit fluorometer (Thermo Fisher).

Commercially synthesised biotinylated 80-mer RNA baits (Arbor Biosciences, MI, USA) were used to enrich the libraries for avian mitochondrial DNA (Mitchell et al. 2014). DNA-RNA hybridisation enrichment was performed according to manufacturer's recommendations (MYbaits protocol, v1 for the ANWC samples and v3 for the Te Papa samples) with the exception that we used 1.25 μL of baits per reaction and changed the incubation step to: 55 °C for 15 h followed by 50 °C for 16 h (for Te Papa samples), or 3 h at 60 °C, 12 h at 55 °C, 12 h at 50 °C, then 17 h at 55 °C (for ANWC samples). The beads were washed three times with 0.1 × SSC and 0.1% SDS solution (5 min, 50 °C) for Te Papa samples, or three times with MYbaits wash buffer v1 (5 min, 55 °C) for ANWC samples. Full-length Illumina sequencing adapters were then added to the enriched libraries via a final round of "off-bead" PCR split into five replicate PCRs (25 μL) containing 1 × Gold PCR buffer, 2.5 mM $MgCl_2$, 1 mM dNTPs, 0.5 μM each primer (IS4 & indexing primer; Meyer and Kircher 2010) and 0.1 U AmpliTaq Gold. Cycling conditions were as follows: 94 °C for 12 min, 15 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 40 s, followed by 72 °C for 10 min. PCR replicates were pooled and purified as before but eluted in 30 μL H_2O and quantified on a TapeStation (Agilent Technologies). Enriched libraries were

pooled, diluted to 2 nM, and run on an Illumina NextSeq 500 (2 × 150 paired end for ANWC samples, 2 × 75 paired end for Te Papa samples).

Bioinformatics

Sequenced reads were demultiplexed using SABRE (https://github.com/najoshi/sabre) via the unique 5′ and 3′ barcodes and processed using the Paleomix pipeline v1.2.12 (Schubert et al. 2014). Within Paleomix, adapter sequences were removed and paired end reads merged using ADAPTER REMOVAL 2.1.7 (https://github.com/MikkelSchubert/adapterremoval). Low quality bases were trimmed (Phred20 – minquality = 4) and overlapping read pairs were merged. Reads shorter than 25 bp were discarded (minlength = 25). Read quality was visualised before and after adapter trimming using fastQC v0.11.5 (https://github.com/chgibb/FastQC0.11.5/blob/master/fastqc). Merged reads were mapped against the mitochondrial Cytochrome *b* gene of *P. solandri* (GenBank accession: KX123188.1) using BWA v0.7.15 (aln -l 1024, seed inactivated; -n 0.01, -o 2; https://github.com/lh3/bwa/releases), with minimum mapping quality set at 25. Consensus sequences were called with Geneious v.10.1.3 (https://www.genious.com), with a consensus threshold set to 85% and minimum depth 4. Sequencing reads from our extraction blank controls could not be mapped to the reference.

Statistical analyses

A phylogeny was built using Cytochrome *b* sequences of modern *P. solandri* samples (n = 176; Lombal et al. 2017) and 22 ancient samples that were successfully sequenced. Homologous data were included from *P. pycrofti* (GenBank accession: MH828447), *P. neglecta* (GenBank accession: U74341), *P. nigripennis* (GenBank accession: U74343), *P. cervicalis* (GenBank accession: EU979553), *Ardenna pacifica* (GenBank accession: AF076088) and *A. carneipes* (GenBank accession: KY443837) given that they breed or are thought to have previously bred on Norfolk Island (Hermes et al. 1986; Holdaway and Anderson 2001). Homologous data from a range of *Pterodroma* species with available Cytochrome *b* sequences on GenBank—*P. brevipes* (GenBank accession: MH828446), *P. leucoptera* (GenBank accession: MK327609), *P. defilippiana* (GenBank accession: MK327608), *P. arminjoniana* (GenBank accession: GQ328986), *P. heraldica* (GenBank accession: GQ328988), *P. cookii* (GenBank accession: U74345), *P. longirostris* (GenBank accession: U74344), *P. hypoleuca* (GenBank accession: AF076079), *P. axillaris* (GenBank accession: U74342) and *Ardenna bulleri* (GenBank accession: AF076081)—were also added to our dataset as a means to assign sequences to other potential candidate contemporary species. Homologous data from *Onychoprion fuscatus* (=*Sterna fuscata*) (GenBank accession: AY631305) was used as an outgroup.

We used Akaike Information Criterion (AIC) scores generated in jModeltest v2.1.10 (Darriba et al. 2012) to identify the best fit candidate model of nucleotide evolution (TVM+I+G). This model was employed in PhyML v3.0 (Guindon and Gascuel 2003) to estimate the maximum likelihood topology, using a BioNJ starting topology and nearest neighbour interchange tree rearrangement. The robustness of the tree was evaluated using 100 bootstrap replicates. A haplotype network was built using the TCS method as implemented in PopART (Leigh and Bryant 2015) for the same data. Estimates of pairwise population differentiation (F_{st}, G_{st}) among Lord Howe Island (modern and ancient samples), Phillip Island and Norfolk Island were determined using SPADS v 1.0 (Dellicour and

Mardulyn 2014). The statistical significance of F_{st} and G_{st} values was assessed based on 10,000 random permutations of individuals among islands.

Results

A total of 22 subfossil samples were successfully sequenced for Cytochrome *b* (720 bp; Table 1). The subfossil samples appeared in three clades in the phylogenetic tree. Eleven of the ancient samples, all morphologically identified as *P. solandri* (five from Lord Howe Island and six from Norfolk Island) formed a clade with the 176 modern *P. solandri* samples (C1 in Fig. 2). The remaining subfossil samples in the phylogeny were all collected on Norfolk Island. Ten subfossil bone samples, including five morphologically identified as *P. pycrofti* (S45699.2, S45701.1, S45705.2, S45707.2, S45709.1) and five morphologically identified as *Pterodroma* spp. (P00126, P00128, P00133, P00134, P00137), formed a clade with three other species of *Pterodroma* (C2 in Fig. 2). The remaining subfossil sample, initially morphologically identified as *P. solandri* (S45710.3), clustered with species of *Ardenna* (C3 in Fig. 2); it is a fragment of a humerus which is morphologically similar to both *P. solandri* and *A. pacifica* but it fits *A. pacifica* better, so it was evidently misidentified originally (A. Tennyson pers. obs. 2018). We do not consider it further in this study.

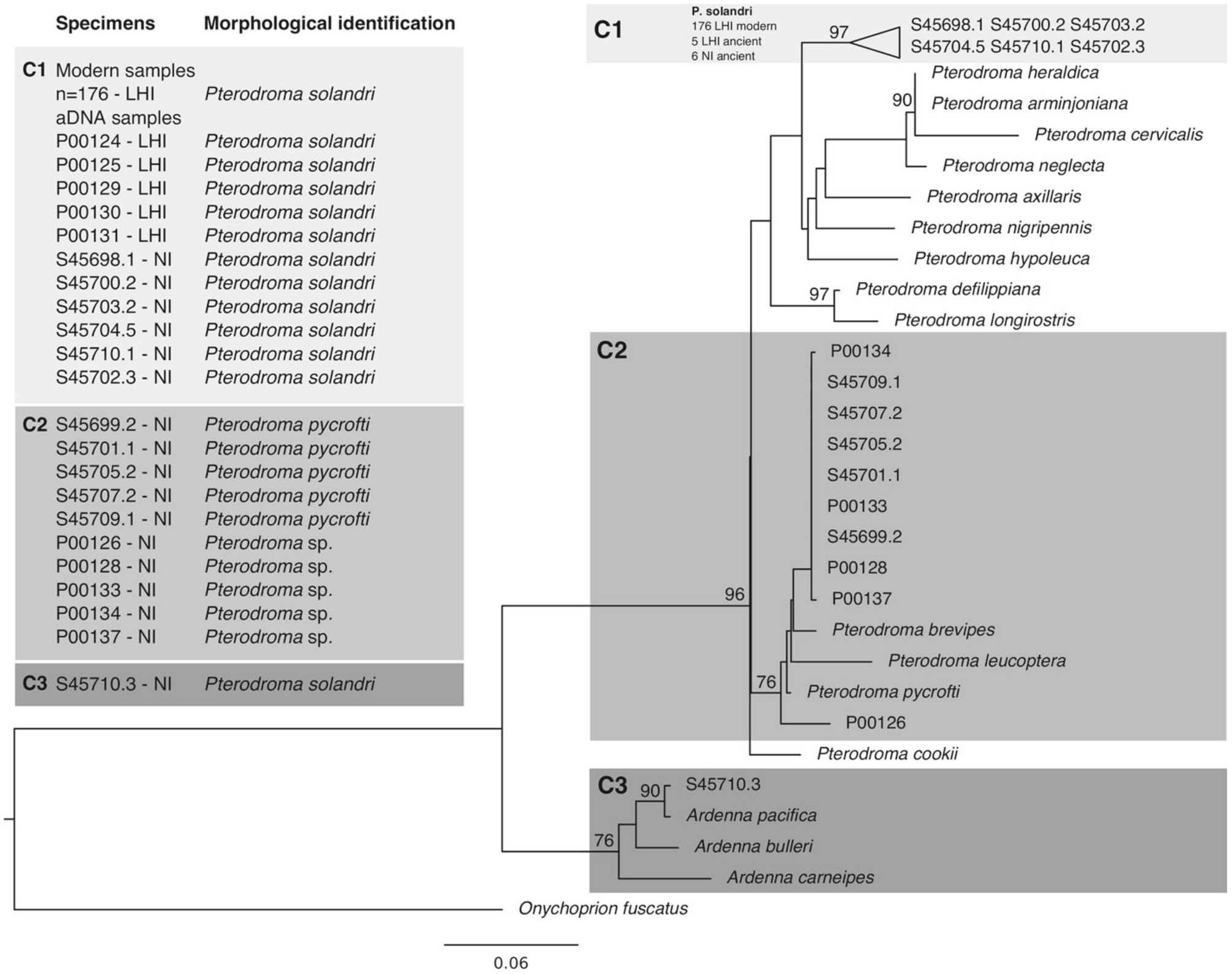

Fig. 2 Maximum Likelihood phylogenetic tree of *Pterodroma* Cytochrome *b* including modern (n = 176) and ancient samples (n = 22, see Table 1) collected in Lord Howe Island and Norfolk Island, respectively. The tree is drawn to scale, with branch lengths measured as the number of substitutions per site. Only bootstrap values > 70% are indicated on the cladogram

A haplotype network was built for 187 *P. solandri* samples (176 modern and 11 ancient samples) including all individuals morphologically identified as *P. solandri* (except the sample affiliated with *Ardenna*; see above). The relationships between the 21 haplotypes are shown in Fig. 3. Four out of the five ancient samples collected on Lord Howe Island (P00124, P00125, P00129, P00131) exhibited the most frequent haplotype observed in modern samples (Hap_6), with the remaining Lord Howe ancient sample (P00130) representing a unique haplotype that differed by one mutation (Hap_21; Fig. 3). Five out of the six ancient *P. solandri* sequences from Norfolk Island (S45698.1, S45700.2, S45703.2, S45704.5, S45710.1) also exhibited Hap_6. One Norfolk Island sample (S45702.3)

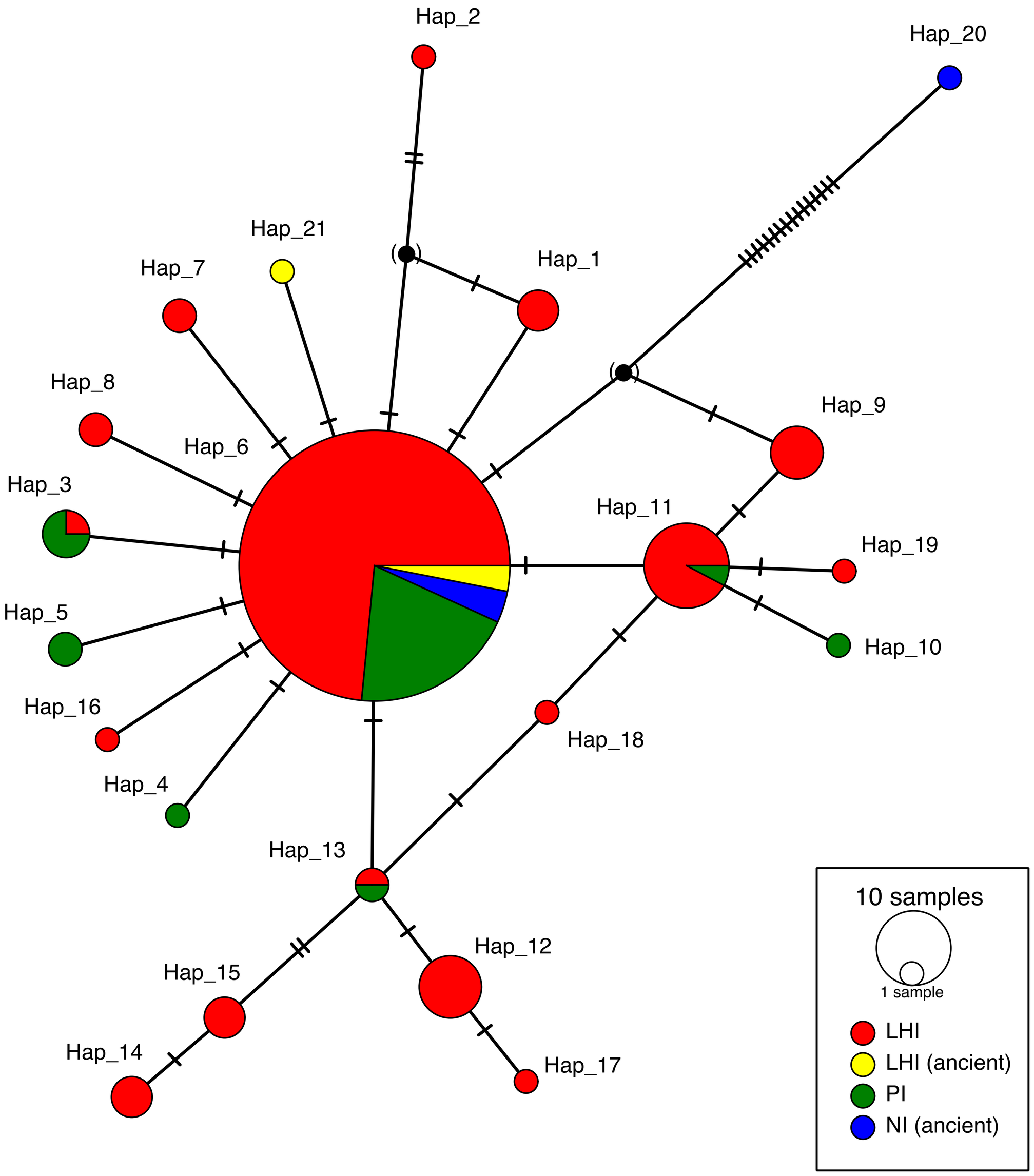

Fig. 3 TCS haplotype network of *Pterodroma solandri* Cytochrome *b* (Clade C1). Haplotypes are represented by circles, where the size of each circle is proportional to the frequency of the corresponding haplotype. Lines on connecting branches represent mutations. Red: modern Lord Howe Island individuals. Yellow: ancient Lord Howe Island individuals. Green: modern Phillip Island individuals. Blue: ancient Norfolk Island individuals

represented a unique haplotype (Hap_ 20) that was separated from haplotype 6 by 17 mutations (Fig. 3). Measures of population genetic structure (F_{st} = 0.017, $p > 0.05$; G_{st} = 0.028, $p > 0.05$) were low and not significant.

An additional haplotype network was built for the second clade C2 including 10 ancient samples (S45699.2, S45701.1, S45705.2, S45707.2, S45709.1, P00126, P00128, P00133, P00134, P00137) and three *Pterodroma* species (*P. pycrofti*, *P. brevipes*, *P. leucoptera*) (C2; Fig. 4). With the exception of P00126, all ancient samples exhibited the same haplotype that was separated from *P. pycrofti* and *P. brevipes* by one mutation (Fig. 4). The specimen P00126 showed a distinct haplotype separated from the other ancient specimens by one mutation (Fig. 4).

Discussion

Connectivity as a buffer of genetic diversity losses from the Norfolk Island extirpation in *P. solandri*

DNA sequences from heterochronous samples consisting of sub-fossil bones from Norfolk Island and Lord Howe Island, and those representing the entire current breeding range of *P. solandri*, are dominated by the same Cytochrome *b* haplotype. This indicates that extirpation of the Norfolk Island colony ascribed to *P. solandri* did not represent a loss of mitochondrial genetic or species diversity. While the loss of the Norfolk Island colony may also represent a large decline for the species as a whole (perhaps up to a 97% decline), the low starting mitochondrial genetic diversity at the time of the loss, documented herein, coupled with the short subsequent time interval and long generation time of the species (15 years; Warham 1990) explains the lack of a temporal change in mitochondrial genetic

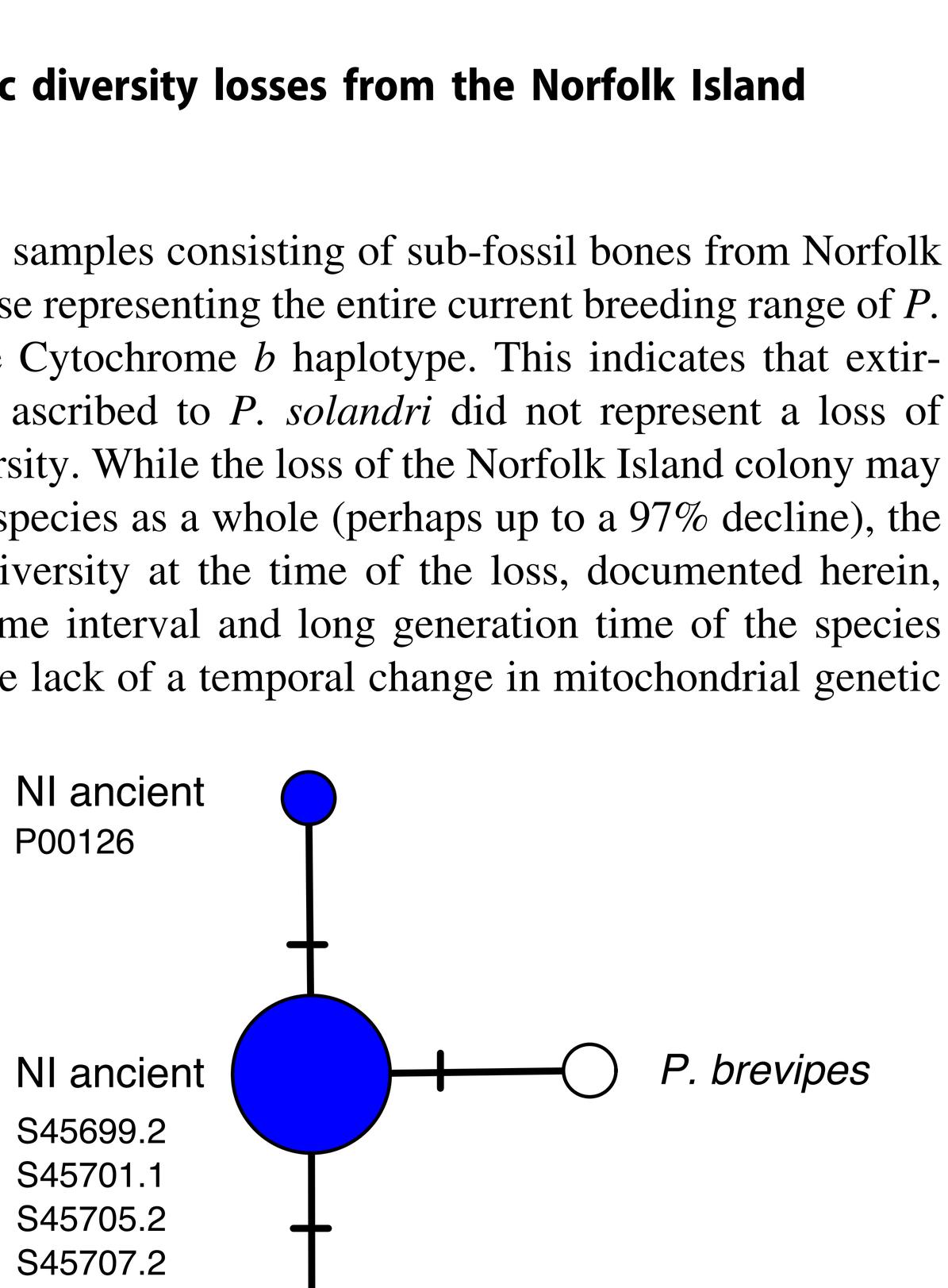

Fig. 4 TCS haplotype network of *Pterodroma* Cytochrome *b* from Norfolk Island (Clade C2). Haplotypes are represented by circles, where the size of each circle is proportional to the frequency of the corresponding haplotype. Lines on connecting branches represent mutations. Blue: subfossil bones from Norfolk Island morphologically identified as *P. pycrofti*

diversity (Welch et al. 2012). Even in instances when a genetically distinct seabird population is extirpated, genetic diversity may be transferred from declining populations to larger colonies, which present greater social attraction for juveniles prospecting for nest sites (Welch et al. 2012). While mtDNA represents one of the most sensitive markers to detect changes in genetic diversity through time, it is only a single, maternally-inherited, locus, and genetic variation of adaptive significance in the nuclear genome may have been lost, although we consider this unlikely, and the species has been able to re-establish on Phillip Island, within the Norfolk group (Lombal et al. 2017).

Several factors such as vagility, large effective population size, and long generation time may have contributed to the absence of genetic differentiation of the *P. solandri* colonies, and hence the lack of genetic consequences from the Norfolk Island extirpation (Frankham et al. 2002). However, the level of connectivity that maintained genetic homogeneity of colonies was insufficient for persistence of populations (Lowe and Allendorf 2010). The population was estimated at ~ 1,000,000 breeding pairs before March 1790 when the HMS *Sirius* was shipwrecked at Norfolk Island. During the next four months, the shipwreck survivors avoided starvation by slaughtering hundreds of thousands of *P. solandri*. An estimated 1600 birds were harvested per night during the four month breeding season, during years 1790 – 1793 (Medway 2002a). The Norfolk population probably was probably extirpated under the influence of subsequent predation and disturbance from invasive mammals such as pigs, which has been documented for other burrowing seabird populations (Hilton and Cuthbert 2010).

Was another *Pterodroma* species historically breeding on Norfolk Island?

One ancient sample morphologically assigned to *P. solandri* did not exhibit the common contemporary *P. solandri* haplotype (S45702.3; Fig. 2) and differed by 17 mutations (Hap_20; Fig. 3). There is no suggestion that this sequence is erroneous – the DNA was treated with UDG prior to library preparation to remove damaged cytosines—and translation reveals no stop codons or amino acid substitutions relative to the other *P. solandri* haplotypes. This sample may represent another species historically breeding on Norfolk Island or a vagrant. Only four *Pterodroma* species are reported to currently breed in the Norfolk group—*P. solandri*, *P. cervicalis*, *P. nigripennis* and *P. neglecta* (see Hermes et al. 1986; Holdaway and Anderson 2001)—and reference sequences from all are represented in our analysis. The sequence did not match with anything else in GenBank. However, the list of breeding species might be incomplete, and it is possible that another unrecognized species bred on Norfolk Island before European settlement (see Appendix in Holdaway and Anderson 2001). Taxonomically cryptic species have been widely observed in insular regions (Murphy et al. 2011; Saitoh et al. 2015). For example, the differences in size and morphology of *Pterodroma* subfossils from the Canary Islands compared with the two current Macaronesian breeding species of *Pterodroma* (Fea's petrel *P. feae* in the Cape Verde Islands and the Madeiran petrel *P. madeira*) indicate the possible existence of a distinct *Pterodroma* in the Canary Islands historically (Rando 2002). Similarly, morphological and genetic evidence revealed the extinct *P. imberi* from the Chatham Islands, which is intermediate in size between two extant species (Cooper and Tennyson 2008; Tennyson et al. 2015).

The Norfolk subfossils morphologically described as *P. pycrofti* analyzed in the present study all showed the same haplotype that differed from *P. pycrofti* and *P. brevipes* by one mutation (Fig. 4). *Pterodroma pycrofti* and *P. brevipes* bones overlap in size (A. Tennyson

pers. obs.). Our genetic results suggest that these subfossils may be *P. pycrofti, P. brevipes*, or another closely-related, possibly undescribed taxon. However, the current breeding distribution of *P. pycrofti* in temperate waters (small islands off the northeastern coast of the North Island of New Zealand; Heather and Robertson 1996) fits better with a breeding population on Norfolk Island than does that of *P. brevipes*, which is restricted to tropical Pacific breeding populations (see Tennyson and Miskelly 2012). Further research should be conducted to address this uncertainty, using nuclear DNA markers and broader population sampling of these two species.

Conclusions

The extirpation of the Norfolk Island population of *P. solandri* did not affect species diversity but ancient DNA analyses of smaller *Pterodroma* bones from Norfolk Island indicate the loss of a possibly undescribed taxon from the Tasman Sea.

Humans have had a great impact on global biodiversity, especially on the islands of the Pacific Ocean (Pimm et al. 1994; Steadman 2006, 1989). Norfolk Island fits the pattern of several other Pacific islands, whereby human intervention resulted in the extirpation of a resident bird population. In this instance, no Cytochrome *b* diversity within *P. solandri* was lost as a consequence of the Norfolk Island extirpation. However, we identified one mtDNA lineage (haplotype 20) in a single subfossil bone and a mtDNA clade (nine subfossil bones) closely related to *P. pycrofti* and *P. brevipes* that have been extirpated from Norfolk Island since the 13th Century. The genetic similarity of extirpated Norfolk and contemporary Lord Howe *P. solandri* supports the likely success of efforts to translocate individuals and re-establish a population on Norfolk Island. This will reduce the extinction risk of *P. solandri* and restore the input of marine-derived nutrients into the ecosystem (particularly phosphorous) with potential benefits for broader ecosystem restoration on Norfolk Island.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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