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Detecting cryptic burrowing petrels recovery post eradication in a remote landscape

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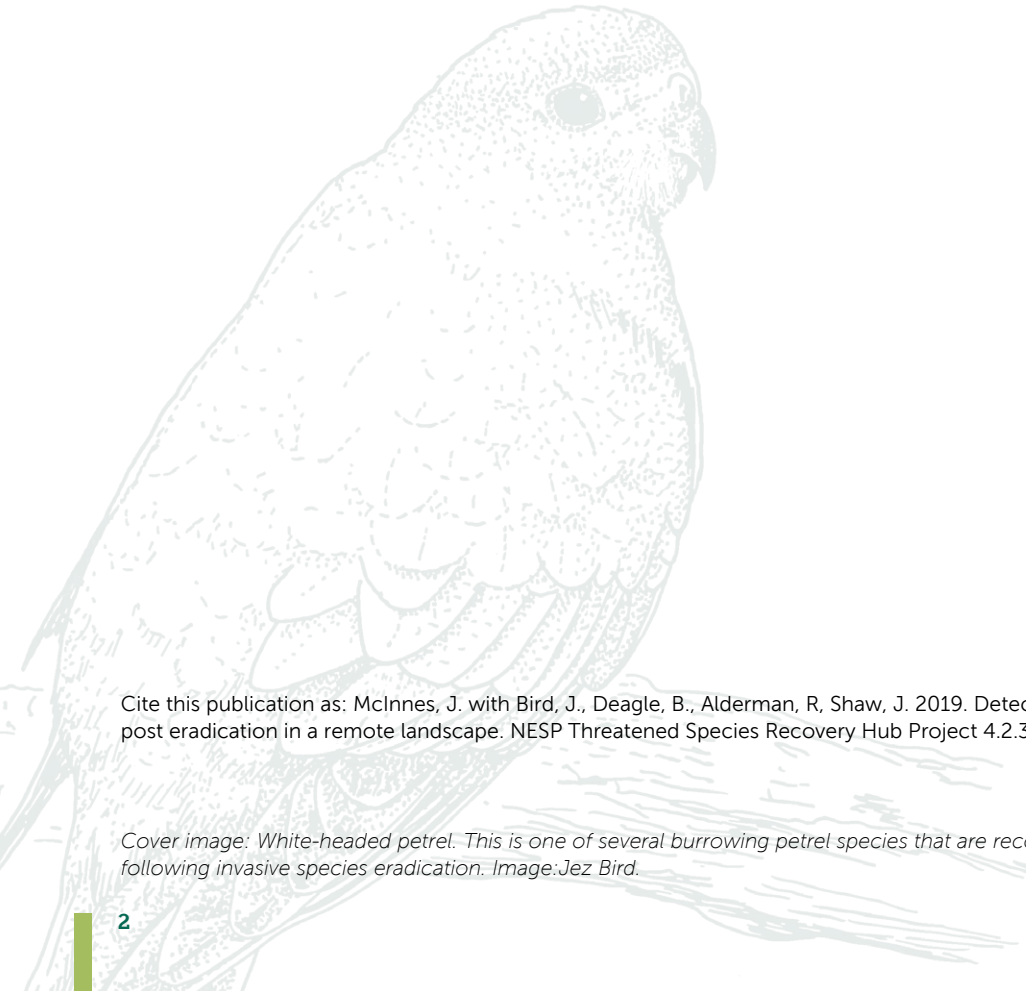

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Cover image: White-headed petrel. This is one of several burrowing petrel species that are recovering on Macquarie Island following invasive species eradication. Image:Jez Bird.

Contents

Executive Summary

Identifying the species diversity of an ecosystem provides valuable insights into the impact of environmental changes, as well as ecosystem recovery. A large proportion of petrels and prions are listed as threatened; however, these species are often cryptic breeders and difficult to study. This case study assessed the value of incorporating DNA methods into field surveys to identify burrowing petrel species and distribution by using scats and feather samples collected near burrows as part of a monitoring program on Macquarie Island. A total of 330 samples were collected, with burrowing petrel DNA identified in 280 samples. Both scats and feather yielded sufficient DNA to identify species using two genetic marker sets. DNA from eight burrowing petrel species was identified across the island, including diving petrels and fairy prions that are rarely detected on the main island. As well as species identification, this study highlighted potential intra-specific genetic differences between the Macquarie island population and voucher specimens previously collected on other sub-Antarctic islands. This study highlights the value of DNA methods to assess species diversity and distribution, and should be incorporated into future burrowing petrel studies to complement ground surveys

Introduction

Species biodiversity assessments are a key component of ecosystem monitoring. These assessments provide valuable information about which species are present and, importantly, whether species diversity changes under different environmental and anthropogenic pressures. There are a suite of tools used to assess species diversity and distribution. These vary across species, landscape and habitats and over time with technological advancements. Some cryptic species are difficult to detect without either an a priori knowledge of which species to expect, or extended monitoring periods.

DNA metabarcoding analysis provides a rapid biodiversity assessment tool that can be used to identify species presence in an ecosystem (Ruppert et al. 2019). This may be achieved through indirect sample collections, such as water or soil (Andersen et al. 2012, Thomsen et al. 2012), or more direct sample collections such as tissue samples (Alonso et al. 2014), scats to identify the defecator (Fernandes et al. 2008) or the prey field (Deagle et al. 2009), or parasites (Calvignac-Spencer et al. 2013).

Petrels and shearwaters are some of the most endangered groups of species globally (Dias et al. 2019). These species face a number of ongoing threats including predation pressure from invasive species, fishery bycatch, and changing environmental conditions through climate change (Dias et al. 2019, Rodríguez et al. 2019). To understand these threats and their impacts on species populations, there is an ongoing need to gather data on species diversity and distribution, and resolve taxonomic uncertainty. Of the Procellariformes, burrowing petrels are particularly difficult to study due to the cryptic nature. Breeding sites are often extremely fragile and remote, and burrows are often too long to identify the occupant (Rodríguez et al. 2019). DNA analysis may provide a fast and cost-effective way to rapidly identify burrowing petrel species in a region, especially when shifts in species diversity are likely to occur.

Macquarie Island and associated offshore stacks have been the recorded breeding site for at least eight burrowing petrel species (Table 1, Brothers 1984, Schulz et al. 2005, DPIW 2007). However, due to the long history of invasive species on the island, there have been considerable changes in species abundance and distribution. Only three species (Antarctic prions, white-headed petrels and sooty shearwaters) commonly bred on the main island until the eradication of cats in 2000. Since then, grey petrels and soft-plumage petrels have been resighted breeding (Schulz et al. 2005, DPIW 2007). By 2014, the remaining invasive vertebrates (rats, mice and rabbits) were also eradicated. In the following years, blue petrels, which were previously restricted to offshore stacks, have also returned to breed on the main island (Bird et al *unpublished data*), however, diving petrels and fairy prions are still rarely detected.

This study uses burrowing petrel scats and feathers to assess the applicability of molecular methods to determine the diversity of cryptic burrowing petrels on a remote sub-Antarctic Island. Burrowing petrel specific markers were developed and applied to scat and feather samples from across Macquarie Island to test the viability of degraded samples for species identification. Using these data, we identify which species are present and map their distribution based on the species detected with DNA.

Table 1: Burrowing petrel species known to breed on Macquarie Island (or offshore islands) and recorded vagrants.

Breeding Species Reference	
Common species	
• Antarctic prion, *Pachyptila desolata*	Brothers (1984)
• Blue petrel, *Halobaena caerulea*	Brothers (1984)
• Grey petrel, *Procellaria cinerea*	Schulz et al. (2005)
• Sooty shearwater, *Ardenna griseus*	Brothers (1984)
• White-headed petrel, *Pterodroma lessonii*	Brothers (1984)
Less common species	
• Common diving petrel, *Pelecanoides urinatrix*	Brothers (1984)
• Fairy prion, *Pachyptila turtur*	Brothers (1984)
• Soft-plumaged petrel, *Pterodroma mollis*	DPIW (2007)
Vagrants	
• Great-winged petrel, *Pterodroma macroptera*	DPIPWE Internal reports, Brothers (1984)
• Grey-backed storm petrel, *Garrodia nereis*	
• Kerguelen petrel, *Lugensa brevirostris*	
• Little shearwater, *Puffinus assimilis*	
• Mottled petrel, *Pterodroma inexpectata*	
• Short-tailed shearwater, *Ardenna tenuirostris*	
• South-Georgian diving petrel, *Pelecanoides georgicus*	
• Snow petrel, *Pagodroma nivea*	
• Slender-billed prion, *Pachyptila belcheri*	
• White-chinned petrel, *Procellaria aequinoctialis*	
• Wilson's storm petrel, *Oceanites oceanicus*	

Methods

Sample collection

Scat and feather samples were collected from Macquarie Island between November 2017 – March 2018, and October–November 2018. Samples were collected from either 1) breeding sites of known species occupancy (study sites), or 2) sites with mixed or unknown species occupancy (exploratory). Samples from group one were predominantly collected from sites with white-headed petrels, blue petrels or Antarctic prions sightings confirmed. Samples for the group two were collected at either North Head, Brothers Point, Green Gorge or during transects across the island. GPS co-ordinates were taken for each sample collected and the date recorded. The freshness of scat samples was recorded as either 'old', when a sample was dry and compacted, or 'recent' when a sample was wet and retained some shape. The pigment of a subset of samples was recorded to test if samples that were all white, and therefore contained predominantly urea, would yield sufficient bird DNA.

Primer design

Burrowing petrel primers were designed for two gene regions, Cytochrome Oxidase 1 (CO1) and the nuclear small subunit 16S gene (16S, Table 2). The CO1 primer set was designed to specifically target burrowing petrels as there is an extensive reference database of sequences for alignment. The 16S primer set was designed to more broadly detect vertebrates due to the low number of burrowing petrel samples available to develop primers. Existing sequences from the families Procellariidae (prions, shearwaters, fulmarine petrels and gadfly petrels), Pelecanoididae (diving petrels), Oceanitidae (southern storm petrels) and Hydrobatidae (northern storm petrels) were downloaded from Genbank and the Barcode of Life Database (BOLD) for the CO1 gene region, and from Genbank for the 16S region. Primers were be tested and optimised with DNA extracted from burrowing petrel flesh samples, as well as a subset of scats and feathers.

PCR Round	Primer Name	Primer sequence (5'-3')	Fragment length	Amp. Temp	Reference
1	CO1_BP_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTCG TAATRATCTTYTTYATRGT	~142bp	54 oC	This study
1	CO1_BP_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCY GCBCCTGCTTCYAC			This study
1	16S_Vert_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAC GAGAAGACCCTRTGGA	~208bp	64 oC	This study
1	16S_ ChorCeph_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATT GCGCTGTTATCCCT			Deagle et al. (2009)
2	SSU3_Tag_F1	AATGATACGGCGACCACCGAGATCTACACAGTTCGGA CTTCGTCGGCAGCGTC			Jarman et al. (2013)
2	SSU3_Tag_R1	CAAGCAGAAGACGGCATACGAGATAGCTTAGGCTGTC TCGTGGGCTCGG			Jarman et al. (2013)

DNA extraction, PCR amplification and amplicon sequencing

DNA was extracted from feathers by the Australian Genome Research Facility (AGRF), a single column nucleic acid extraction was carried out using the base of the shaft of each feather. DNA was extracted from scat samples and flesh samples using a Promega 'Maxwell 16' instrument and a Maxwell® 16 Tissue DNA Purification Kit. PCR inhibitor concentrations were diluted by mixing a small amount (~30mg) of the faecal samples in 250ul of STAR buffer (Roche Diagnostics) prior to extraction.

Each sample was amplified with both the CO1 and 16S markers. PCR reactions for each primer set were carried out separately as a two stage process. Stage one PCR reactions (10 µL) were performed with 5 µL 2 x Phusion HF (NEB), 0.2 µL 100 x Bovine Serum Albumin (NEB), 0.7 µL 10 µM of each CO1 amplification primer or 0.2 µL 10 µM of each 16S amplification primers (Table 2), 0.5 µL of Evagreen, 1 µL faecal DNA and 1.9 µL (CO1) or 2.9 µL (16S) of water. Thermal cycling conditions were 98°C, for 2 mins; followed by 40 cycles (CO1) or 35 cycles (16S), of 98°C for 5 s, 54°C (CO1) or 64°C (16S) for 20 s, 72°C for 20s, with an extension of 72°C for 1 min. Each sample was run on a LightCycler 480 (Roche Diagnostics). A negative control containing no template DNA and positive control were included in each PCR amplification run. In each reaction the negatives did not amplify and the positives successfully amplified. PCR product from each sample were diluted 1:10 for the second stage PCR. In the second stage PCR, a unique tag was attached to each sample (Table 2). PCR reactions (10 µL) were performed with 5 uL 2 x Phusion HF (NEB), 1 µL of water, 1 µL of 1 µM of each tag primer, and 2 µL of diluted PCR product from stage one. Thermal cycling conditions were 98°C, for 2 min; followed by 10 cycles of 98°C for 5 s, 55°C for 20 s, 72°C for 20 s, with an extension of 72°C for 1 min. Samples were pooled and purified from unincorporated reaction components by washing, utilising reversible binding to Ampure (Agencourt) magnetic beads, with 1.2 µL of Ampure per microlitre of DNA product. Sequencing of PCR products was performed at the Menzies Institute for Medical Research, using an Illumina Miseq high throughput sequencer using the MiSeq reagent kit V2 (300 cycles).

Bioinformatics

Amplicon pools were de-multiplexed based on unique 10 bp Multiplex IDentifiers (MIDs) incorporated in the Illumina two-step MID protocol. Fastq files were processed using USEARCH v11.0.667 (Edgar 2010). Reads R1 and R2 from the paired end sequencing were merged using the fastq_mergepairs function, retaining only merged reads flanked by exact matches to the primers, and primer sequences were trimmed. Reads from all samples were pooled and dereplicated, then clustered into Operational Taxonomic Units (OTUs). To identify unique read sequences, we generated zero-radius OTUs (ZOTUs) using the unnoise3 command. Reads for each sample were assigned to these ZOTUs and a summary table generated using a custom R script.

The ZOTUs derived from the CO1 markers were aligned with the references sequences downloaded from BOLD and Genbank, and a phylogenetic tree was constructed using MEGA-X as a fast way to identify bird sequences compared to other non-bird sequences that were amplified (such as unicellular organisms, prey and plant DNA). ZOTUs that were clustered with birds were assigned to species using the Genbank online database with the Basic Local Alignment Search Tool (BLAST), and against the BOLD database. When a sequence matched multiple species or did not exactly match a species, this was recorded. The proportion of sequences matching each species were calculated for each sample. A sample was assigned to a species if >80% of sequences matched that ZOTU. Due to the poor reference library for the 16S region, the species assignment for each 16S ZOTU was based on CO1 results for those samples. Samples amplified with both markers were included if they contained at least 100 sequences of DNA.

White-headed petrel. Most of the time petrels are at sea, only returning to land by cover of darkness during the breeding season. This, together with their burrow-nesting habit, makes them challenging to survey and monitor, so novel methods are required. Image: Jez Bird

Phylogenetic trees

Phylogenetic trees were constructed using MEGA X (Kumar et al. 2018), reference sequences from the barcode of Life Database (BOLD), and ZOTUs generated from the sequence processing. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993). The tree with the highest log likelihood for each group of species (-274.20) was generated for the genus *Pachyptila, Pelecanoides*, and a subsection of *Pterodroma* that includes *P. mollis* and *P. lessonii*. As per the MEGA X workflow, the percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.

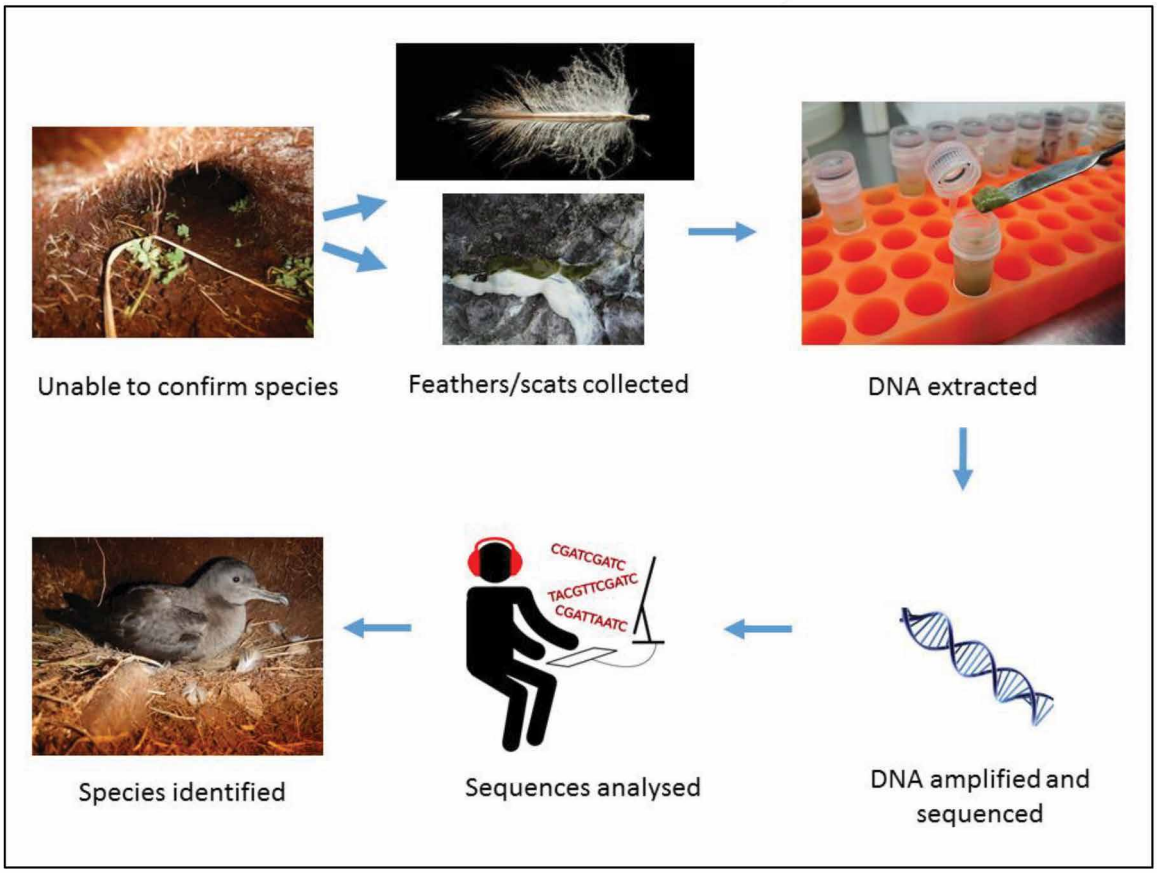

Figure 1: Workflow for DNA detection of scats and feathers

Results

Amplification success

A total of 330 samples were collected, including 222 scat samples and 108 feather samples. Avian DNA was successfully amplified in 303 samples using both markers, comprised of 209 scat samples (94%) and 94 feather samples (87%, Table 3). Both markers amplified the feathers relatively consistently with six feather samples amplified by only a single primer set. Scat samples showed more variability in amplification success between the markers, with 30 samples amplified by only one marker (Table 3). This variability was likely due to the specificity of the primers and the detection of other non-bird DNA in scat samples (e.g. parasites, unicellular and food DNA).

There was no significant difference in the amplification success of feathers and scats for either the CO1 marker X^2= 0.331, p>0.05, or the 16S marker X^2= 0.817, p>0.05 (Table 3). Overall, 91% of scat samples that were classified as 'old' (n=123) amplified bird DNA, compared to 100% of the 'recent' samples (n=79). For the subset of samples where colour was recorded, 86% of samples that were all white (n=30) amplified bird DNA compared to 100% of samples with some pigment (n=47). These white samples were also usually runnier and harder to collect.

Of the 303 samples where bird DNA was detected, 23 samples (19 scats and 4 feathers) weren't from burrowing petrel species. This included twelve samples from *Sturnus vulgaris* (common starling), seven samples from *Stercorarius sp.* (skua), two from *Phoebetria palpebrata* (light-mantled albatross), one from *Larus dominicanus* (kelp gull) and one *Acanthis flemmea* (common redpoll). These 23 samples were excluded from further analysis, giving a total of 280 samples with burrowing petrel DNA detected. The species confirmation with CO1 markers enabled identification of nine new 16S sequences (see Supplementary Material), which can be uploaded to Genbank to enable future dietary and species identification work.

Table 3: Amplification success of scat and feather samples using both the CO1 and 16S markers.

	Number of samples		Total samples	Proportion of samples		Overall prop
N samples amplified bird	**Feather**	**Scat**		**Feather**	**Scat**	
Both markers	88	179	267	81.5%	80.6%	80.9%
CO1 only	4	16	20	3.7%	7.2%	6.1%
16S only	2	14	16	1.9%	6.3%	4.8%
Did not amplify	14	13	27	13.0%	5.9%	8.2%
Total samples	**108**	**222**	**330**			
N samples burrowing petrel detected	90	190	280	83.3%	85.6%	84.8%
N samples other bird detected	4	19	23	3.7%	8.6%	7.0%
Total samples with bird DNA	**94**	**209**	**303**	**87.0%**	**94.1%**	**91.8%**

Species detection and taxonomy

DNA was detected from all eight burrowing petrel species previously recorded breeding on Macquarie Island (Table 4). For seven of these species there was a clear match with reference sequences, but diving petrels could only be assigned to genus (*Pelecanoides*) and one prion sequence was unclear.

One ZOTU closely matched both South-Georgian diving petrels (*P. georgicus*) and common diving petrels (*P.urinatrix*), with only one base pair different between all three sequences and therefore species could not be determined with confidence (Appendix 1B).

Although two samples were confirmed to be fairy prion, there was difficulty distinguishing between fulmar prion (*Pachyptila crassirostris*) and fairy prion (*P. tutur*) DNA in two other samples. The ZOTUs detected in these two samples had a 99% match to fairy prion and fulmar prion (Appendix 1A). Most likely they were all from fairy prions as the samples were collected in close proximity to each other, however cannot be confirmed for these two samples.

Interestingly, the two ZOTUs generated from white-headed petrel (*Pterodroma lessonii*) samples were either an exact match, or one base pair different, to a sequence from Auckland Islands. However, the other six white-headed petrel sequences from Antipodes Island were only a 97% match, with four base pairs different to Macquarie Island samples (Appendix 1C). To put this into context, in this 140bp region the Macquarie Island samples were as different to those from Antipodes as to another species, the great-winged petrel (Pterodroma macroptera), suggesting that the white-headed petrel populations could be distinctly different. However a longer DNA fragment would be needed to test this further.

The ZOTUs that matched Antarctic prions (*Pachyptila desolata*), sooty shearwaters (Ardenna grisea) and grey petrels matched respective reference sequences online, therefore no phylogenetic trees are displayed. There may be genetic differences between the Macquarie Island population of these species and other sites, but this was not evident in this dataset for the CO1 region.

Although there was no difference in the amplification success of scat and feather DNA, there was a difference in the proportion of each sample collected for some species. Feathers were more commonly collected for blue petrels and soft-plumage petrels, but scats were more common for the other species (Table 4).

Table 4: Burrowing petrel species detected using DNA analysis of scat and feather samples on Macquarie Island from November 2017–March 2018 and October–November 2019.

Family	Common Name	Species	Feather	Scat	Total Samples
Species match	Antarctic Prion	*Pachyptila desolata*	30	41	71
	Blue petrel	*Halobaena caerulea*	44	17	61
	Fairy prion	*Pachyptila turtur*		2	2
	Grey petrel	*Procellaria cinerea*		6	6
	Soft-plumage petrel	*Pterodroma mollis*	5	1	6
	Sooty shearwater	*Ardenna grisea*		46	46
	White-headed petrel	*Pterodroma lessonii*	3	55	58
Species unconfirmed	Diving petrel species	*Pelecanoides sp.*	8	20	28
	Fulmar Prion	*Pachyptila crassirostris*		2	2

Species distribution

Targeted sample collections from established study sites resulted in the detection of DNA from all five species that were identified in ground searches (Table 5). These results provided some confidence in the survey method. Exploratory sample collections during island transects and focused area searches detected these five common species, three rarer species and the winter breeding grey petrel (Table 6, Figure 2).

Table 5: Samples collected from study sites to test for any difference in species detection between ground searches and DNA analysis.

Species detected			
Study Site	**Ground search**	**DNA**	**N samples**
Caroline Pt	Blue petrel	Blue petrel	13
		Sooty shearwater	3
Douglas Point	Blue petrel	Blue petrel	5
Douglas Ridge	White-headed petrel	White-headed petrel	2
	Sooty shearwater	Sooty shearwater	*2*
Hill 291	White-headed petrel	White-headed petrel	17
Sodomy Ridge	White-headed petrel	White-headed petrel	13
		Sooty shearwater	1
Square Lake	Antarctic prion	Antarctic prion	12
	White-headed petrel	White-headed petrel	3

Antarctic prions (*Pachyptila desolata*) were distributed across the plateau, and interestingly also on the coastal slopes above Waterfall Bay hut. While these coastal slopes are not typical prion habitat, it was noted during ground surveys that the slope covered in *Acaena* spp. did resemble a prion breeding site; however, no birds were detected.

White-headed petrels (*Pterodroma lessonii*) were found across the plateau and both eastern and western coastal slopes, which was consistent with previous ground surveys.

Sooty shearwaters (*Ardenna grisea*) were found along both eastern and western coastal slopes and one sample was collected from Caroline Point. Although this species is not regularly surveyed, they are known to breed across most of the coastal slopes and were regularly found during this survey in sites with white-headed petrels. These species were not targeted during any of the ground surveys, yet were regularly detected and had the widest coastal distribution (Table 6).

Blue petrels (*Halobaena caerulea*) were detected on lower lying stacks or slopes around the coastline.These included North Head, the connected rock stack at Brother Point, Douglas Point, Caroline Point, Langdon Point stack, West Rock and the inland slopes at Green Gorge.

Soft-plumage petrel (*Pterodroma mollis*) DNA was detected in two samples from Brothers Point, three from Green Gorge, two from West Rock and Langdon Point stack. This is the first time that soft-plumage petrels have been detected at Green Gorge, West Rock and Langdon Point, however, birds have regularly been seen around Brother Point in low numbers.

Diving petrels were detected in five locations around the island: the slopes at the southern end of Hurd Point beach, the southern end of Secluded Bay on North Head, rockstacks at West Rock, Mawson Point and Langdon Point (Figure 2).

Fairy prions (*Pachyptila turtur*) and potentially fulmar prions (*Pachyptila crassirostris*) were only on rockstack areas at Langdon Point, Mawson Point and West Rock. These are the first recent record of fairy prions on the main island. The previous sighting was by Nigel Brothers at an Antarctic Prion site on the plateau in the 1980s.

Grey petrels (*Procellaria cinerea*) were only detected on North Head; however, sample collections were predominantly over summer and therefore only overlapped with the very start of the breeding season (March). North Head is the main breeding site for grey petrels on the island.

Table 6: Distribution of burrowing petrel species grouped by broad location across Macquarie Island. The X corresponds to the detection of that species in at least one sample. Sample sizes were not included here as search effort was different at each site and therefore sample sizes don't reflect abundance.

Common Name	Species	Brothers Point	Eastern Escarpment	Western Escarpment	Green Gorge	Hurd Point	Langdon Point	Mawson Point	Caroline point	North Head	West Rock	Plateau
Antarctic prion	*Pachyptila desolata*		X		X							X
Blue petrel	*Halobaena caerulea*	X			X		X		X	X	X	
Diving petrel	*Pelecanoides sp.*					X	X	X		X	X	
Fairy prion	*Pachyptila turtur*						X				X	
Fairy/fulmar prion	*Pachyptila turtur/crassirostris*							X			X	
Grey petrel	*Procellaria cinerea*									X		
Soft-plumage petrel	*Pterodroma mollis*	X			X		X				X	
Sooty shearwater	*Ardenna grisea*	X	X						X	X		
White-headed petrel	*Pterodroma lessonii*		X	X		X						X

Figure 2: Sampling locations on Macquarie Island in 2017–18 where burrowing petrel species identification was confirmed using DNA from scats and feathers. NB: we couldn't distinguish between fulmar and fairy prion DNA in two samples.

Discussion

DNA was successfully amplified from scats and feathers around Macquarie Island to detect burrowing petrel species diversity, and gave valuable insights into species distribution around the island. Of particular excitement was the detection of fairy prion DNA in several samples at Green Gorge and diving petrel DNA in samples on coastal rock stacks and points, and diving petrel DNA in samples from five locations along the island.

New locations of some rarer species were detected, and some sites were confirmed to be mixed species (table 4). Fairy prion DNA was detected from three coastal rockstacks and diving petrel DNA from five locations around the island. Diving petrels and fairy prions are rarely detected on Macquarie Island, with diving petrels only ever recorded on two offshore rockstacks (Brothers 1984). We detected fairy prions at two new locations (Mawson's Point and West Rock) and one known site (Langdon Point rock stack). Langdon Point rock stack and West Rock were 'hotspots' of burrowing petrel activity, with at least four burrowing petrel species detected at each site. Langdon Point rock stack most likely provided a refuge to burrowing petrel species while rats, mice and rabbits existed on Macquarie Island, as it is not connected to the main island. However, the presence of four petrel species on West Rock, which was previously accessible to invasive vertebrates, is encouraging and provides the first evidence in recent years of fairy prions on the main island.

There was some difficulty differentiating between fairy prion and fulmar prion DNA, and between South Georgian and common Diving Petrels DNA. These two species for each group are very similar both morphologically and genetically. Further work is required both through genetics and on-ground surveys to confirm which species are present. To get species confirmation genetically, either a longer region of the CO1 gene or a different gene region would be needed. The Cytochrome b gene region would be a good option as this has successfully been used to assess genetic differences within and between petrel and prion species (Moodley et al. 2015, Silva et al. 2016), including *Pachyptila* species (Masello et al. 2019). However, there are few reference sequences available for fulmar prions online; therefore museum specimens or targeted sampling at other known breeding locations would be required for any future genetic studies.

The genetic difference between white-headed petrels at Macquarie Island and those at Antipodes Island matches previous observations of morphological differences (Wood et al. 2017). Wood found that white-headed petrels from Antipodes were smaller than Macquarie Island birds in both wing length and culmen length. It is unknown how similar these species are genetically, but these initial results indicate that birds from the two sites have similar differences to those of white-headed petrels and another species, the great-winged petrel. Further work with samples from Macquarie Island, Antipodes Island, Kerguelen and the Auckland Islands may shed more light on the genetic structure of these populations. This current study has built up an excellent white-headed petrel sample base (n=55 samples), however, as these are mostly scat samples, it is unknown how well larger DNA amplicons will amplify. The markers in this study were specifically designed to amplify short DNA fragments, which is ideal for degraded scat samples, but further test will be needed to assess the quality of the DNA for population genetics work.

During this pilot study, both feathers and scats provided high quality DNA, and collection of both sample types would be the best way to proceed in future DNA studies. As there was a difference in the availability of sample types for different species, the collection of just one sample type may bias species detection. Other factors that may bias species detection were the marker choice. The variability in species detection between the 16S and CO1 markers for scat samples was likely to be due to the specificity of the makers. The CO1 markers were designed to target burrowing petrel DNA, whereas the 16S markers detected vertebrates more generally. Consequently, where the CO1 markers didn't detect some other bird species (such as starlings), they did detect burrowing petrels more often than the 16S markers, whereas the 16S markers amplified more non-target DNA, such as DNA from humans and prey, and consequently reduced the proportion of burrowing petrel DNA. Any future work using these methods for burrowing petrel detection could just use the CO1 markers, which have better specificity and reference database. However, the 16S markers would be more useful for more general detection of birds, such as for diet studies; however the reference library would need to be improved.

Although DNA sampling only gives an indication of species presence rather than confirmation of breeding activity, this technique provides a valuable tool to complement other methods to assess burrowing petrel presence. This work highlights the value of DNA methods to confirm species identity where birds cannot be identified, as well as an initial pilot study to identify species presence and provide key locations for follow up census work. The samples also provide good quality DNA and may provide scope for further studies using other markers to look at population genetics of burrowing petrels on Macquarie Island. This case study has shown that using these methods on other remote islands that support cryptic species, such as Heard Island, would be beneficial for any future expeditions.

Future work

- Incorporate DNA sampling into burrowing petrel monitoring as an initial rapid assessment tool to investigate species diversity across island ecosystems.
- Collect both scats and feather samples of all species during island surveys to ensure there is no species bias.
- Use the CO1 markers if focusing specifically on burrowing petrels; otherwise build up a more comprehensive library of burrowing petrel DNA for the 16S region.
- Use another marker (e.g. Cytochrome B) or a longer CO1 amplicon to resolve the species identity of diving petrel and fairy prion/fulmar prion on the island.
- Investigate the genetic structure of white-headed petrel populations.

Supplementary Material

Supplementary Material - Field data and species results.

Acknowledgements

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White-headed petrel captured during burrow inspections to monitor breeding numbers and success on Macquarie Island. Image: Jez Bird

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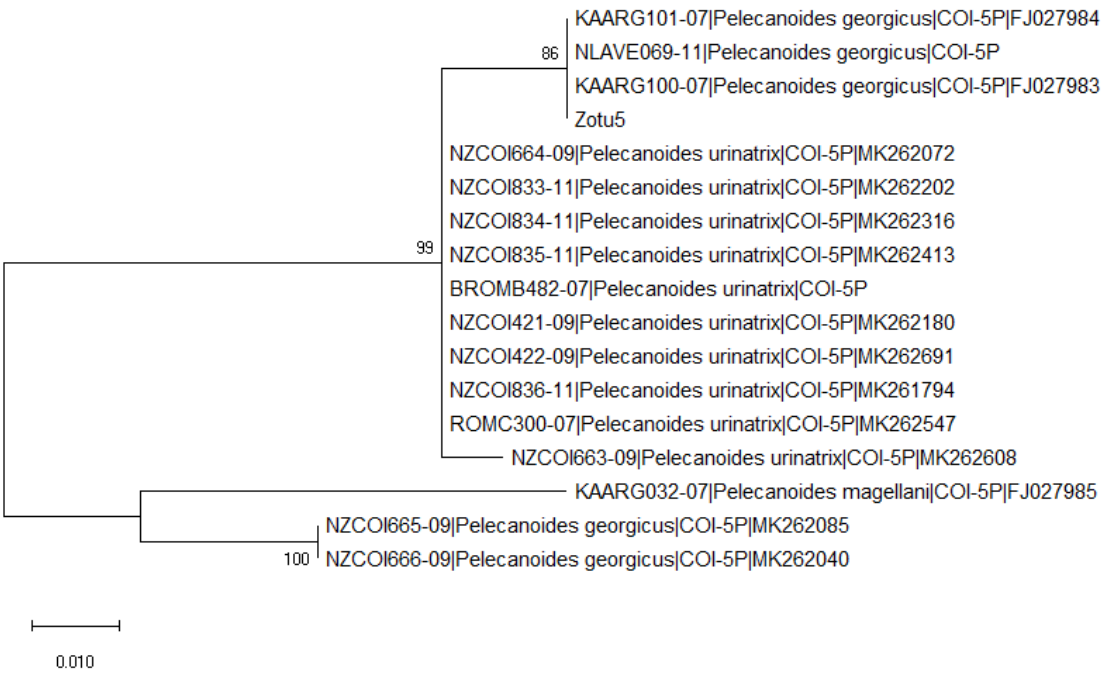
Julie McInnes and Jez Bird conducting fieldwork on Macquarie Island. Image: Toby Travers

Appendix 1 – Phylogenetic Trees

Phylogenetic trees were developed using MEGA X (Kumar et al. 2018) for *Pelecanoides, Pachyptila* and *Pterodroma*. Sooty shearwater and grey petrel ZOTUs matched the reference sequences exactly and therefore trees were not generated. As per the MEGA X workflow, the percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 142 positions in the final dataset for each genus.

A. Pelecanoides – diving petrels

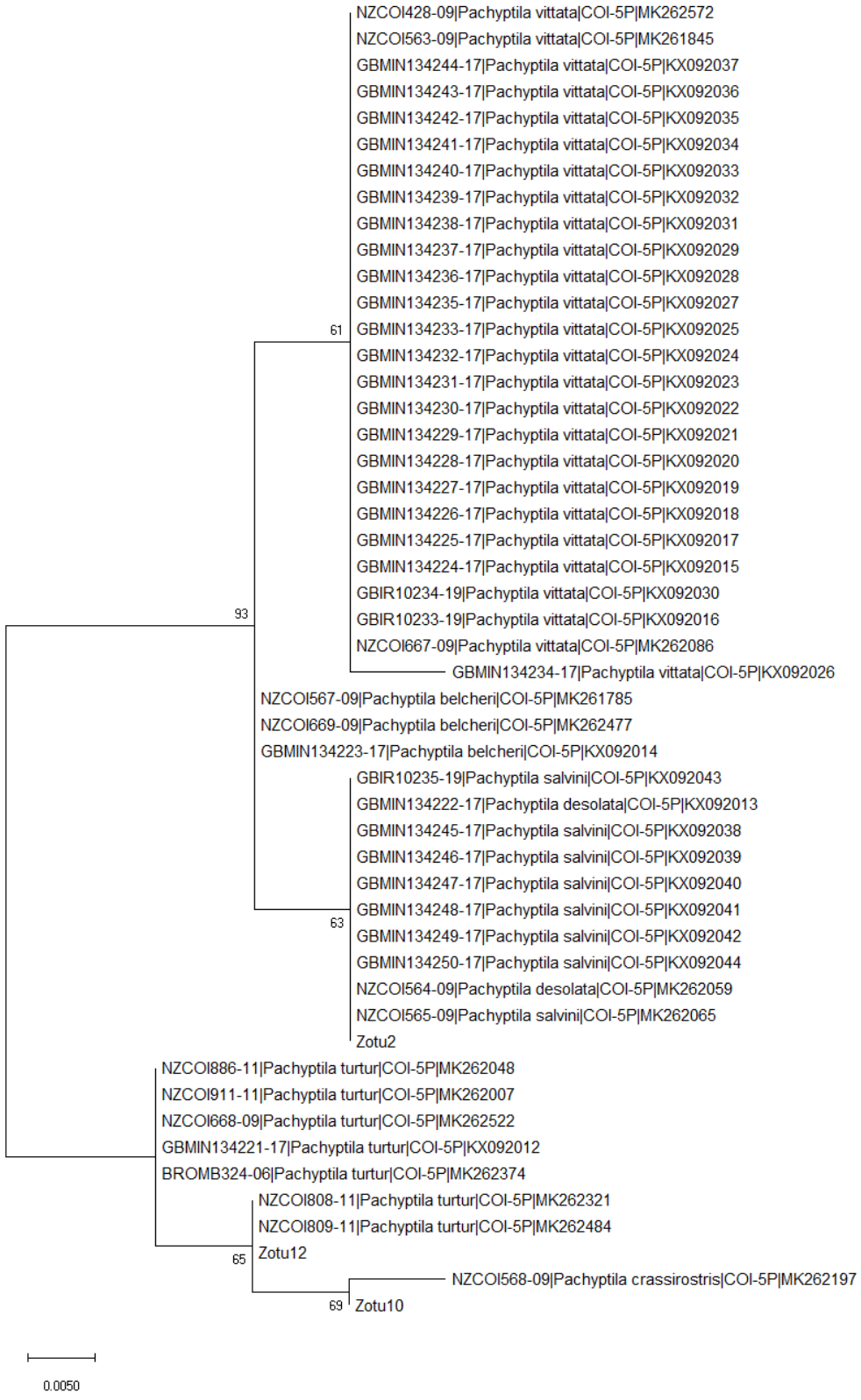
This analysis involved a total of 17 nucleotide sequences, this included 16 reference sequences derived from three species on the BOLD database and one ZOTU (5) that was derived from sequence data analysed in this study.



This blue petrel was captured by a remote camera positioned by the entrance to its nesting burrow. As well as genetic research, other methods for remote monitoring of seabird populations have been trialled at Macquarie Island. Image: Jez Bird

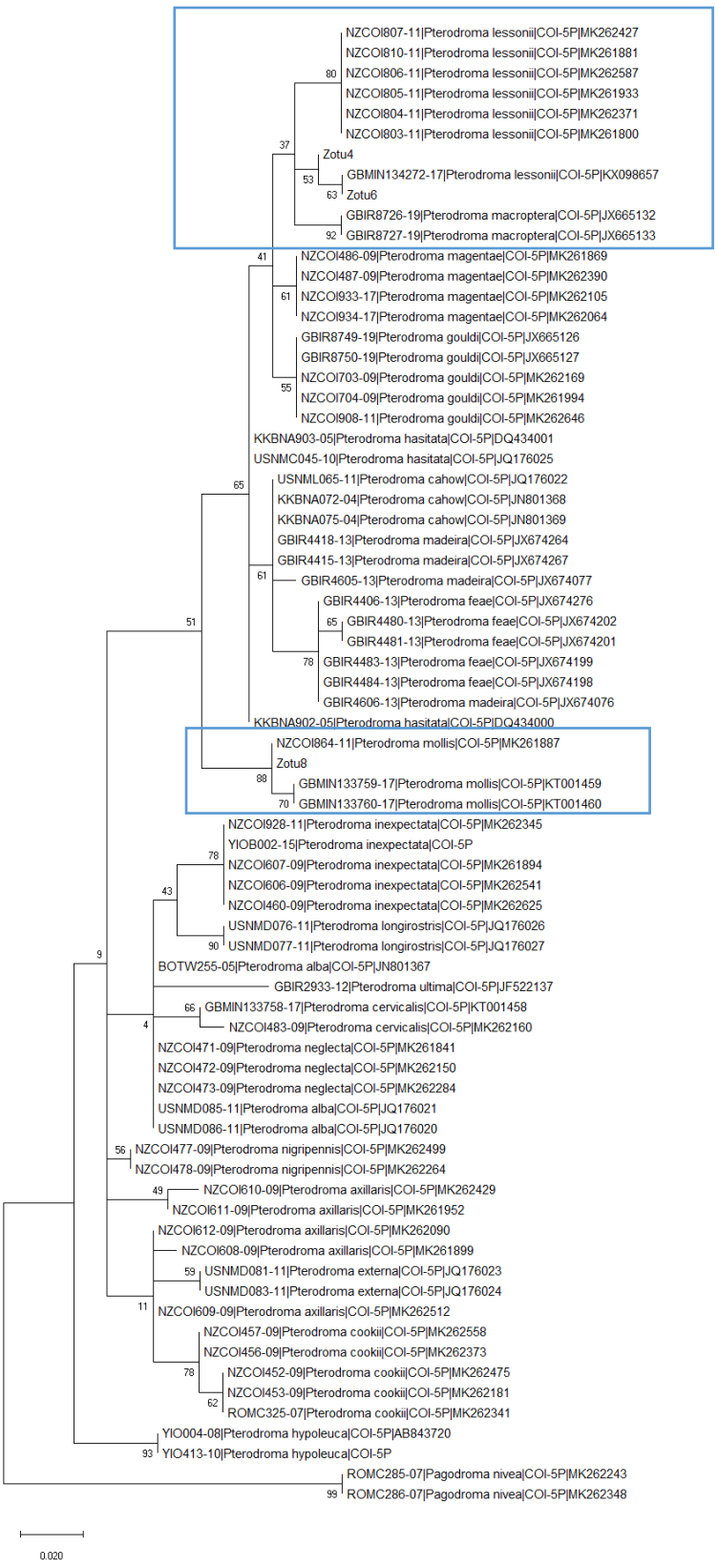
B. Pachyptila – prions

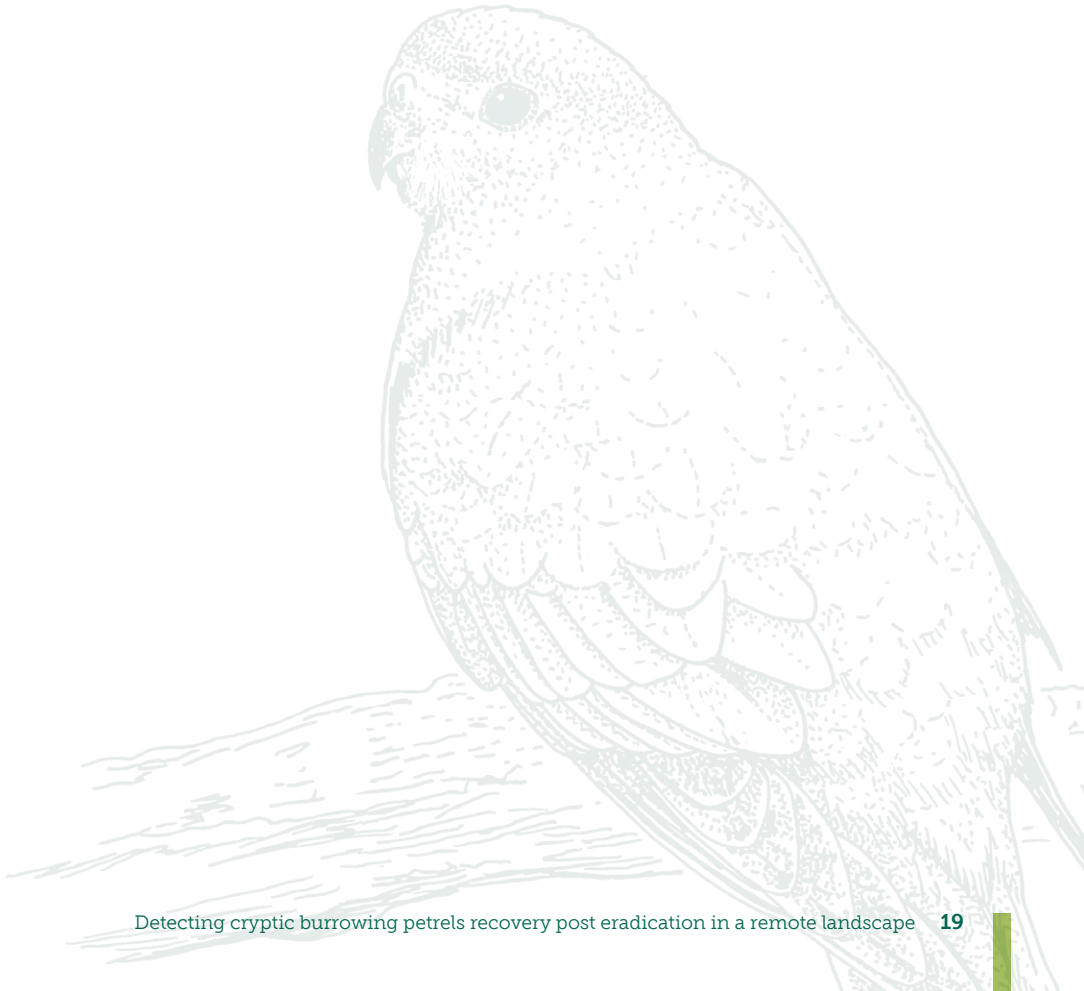
This analysis involved a total of 50 nucleotide sequences, this included 47 reference sequences from six *Pachyptila* species derived from the BOLD database and three ZOTU (2, 10 and 12) that were derived from sequence data analysed in this study.



C. Pterodroma – gadfly petrels (subset)

This analysis involved a total of 74 nucleotide sequences, this included 71 reference sequences from *Pterodroma* species derived from the BOLD database and three ZOTU (4, 6 and 8) that were derived from sequence data analysed in this study.





Further information:
http://www.nespthreatenedspecies.edu.au

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Threatened Species Recovery Hub

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