Received: 27 October 2020 | Revised: 10 April 2021 | Accepted: 11 April 2021

DOI: 10.1111/csp2.439

Conservation Science and Practice
A journal of the Society for Conservation Biology
Open Access
WILEY

PERSPECTIVE

# Using DNA metabarcoding to detect burrowing seabirds in a remote landscape

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**Funding information**
Australian Antarctic Science Grant, Grant/Award Number: AAS 4305; Australian Government National Environmental Science Programme

## Abstract

Species inventories and biodiversity assessments are critical to conservation. Yet cryptic species or recolonizing species can be challenging to detect. DNA metabarcoding provides an alternative tool to identify species that can be difficult to observe during field surveys. We test the efficacy of DNA analysis to identify burrowing petrel species in a rapidly changing landscape, on a remote sub-Antarctic island following pest eradication. Discarded feathers and scats provided high quality DNA for species identification, assisting in detection of new species arrivals and new breeding sites across Macquarie Island. We highlight how DNA metabarcoding informs species inventories and is a valuable tool to complement seabird field surveys.

**KEYWORDS**

ecosystem recovery, eDNA, eradication, faces, noninvasive, *Pelecanoides*, Procellariidae

## 1 | INTRODUCTION

Species diversity assessments are a key component of ecosystem monitoring (Duelli & Obrist, 2003), and understanding of species distributions is required for informed conservation. Monitoring approaches vary across species, landscapes, and habitats. The suite of available methods has grown rapidly in recent years with technological advancements increasing capacity and scope of biodiversity surveys. Yet, despite emerging technologies, some species remain difficult to detect due to their habitat and behavior. Even with considerable prior knowledge and sustained monitoring effort, they remain elusive (e.g., Suryawanshi, Khanyari, Sharma, Lkhagvajav, & Mishra, 2019). DNA metabarcoding provides a rapid assessment tool to identify species presence (Pompanon et al., 2012; Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012), with feathers and scats providing noninvasive DNA samples (Dalen, Götherström, & Angerbjörn, 2004; Rudnick, Katzner, Bragin, & DeWoody, 2007). Here, we explore low-impact genetic sampling to survey burrowing petrels on remote sub-Antarctic

Macquarie Island where their numbers and possibly diversity are expected to increase following vertebrate pest eradication (Figure 1; DPIW, 2007). Our DNA metabarcoding analysis of petrel scats and feathers helped inform species inventories and highlights the value of incorporating molecular methods into field surveys.

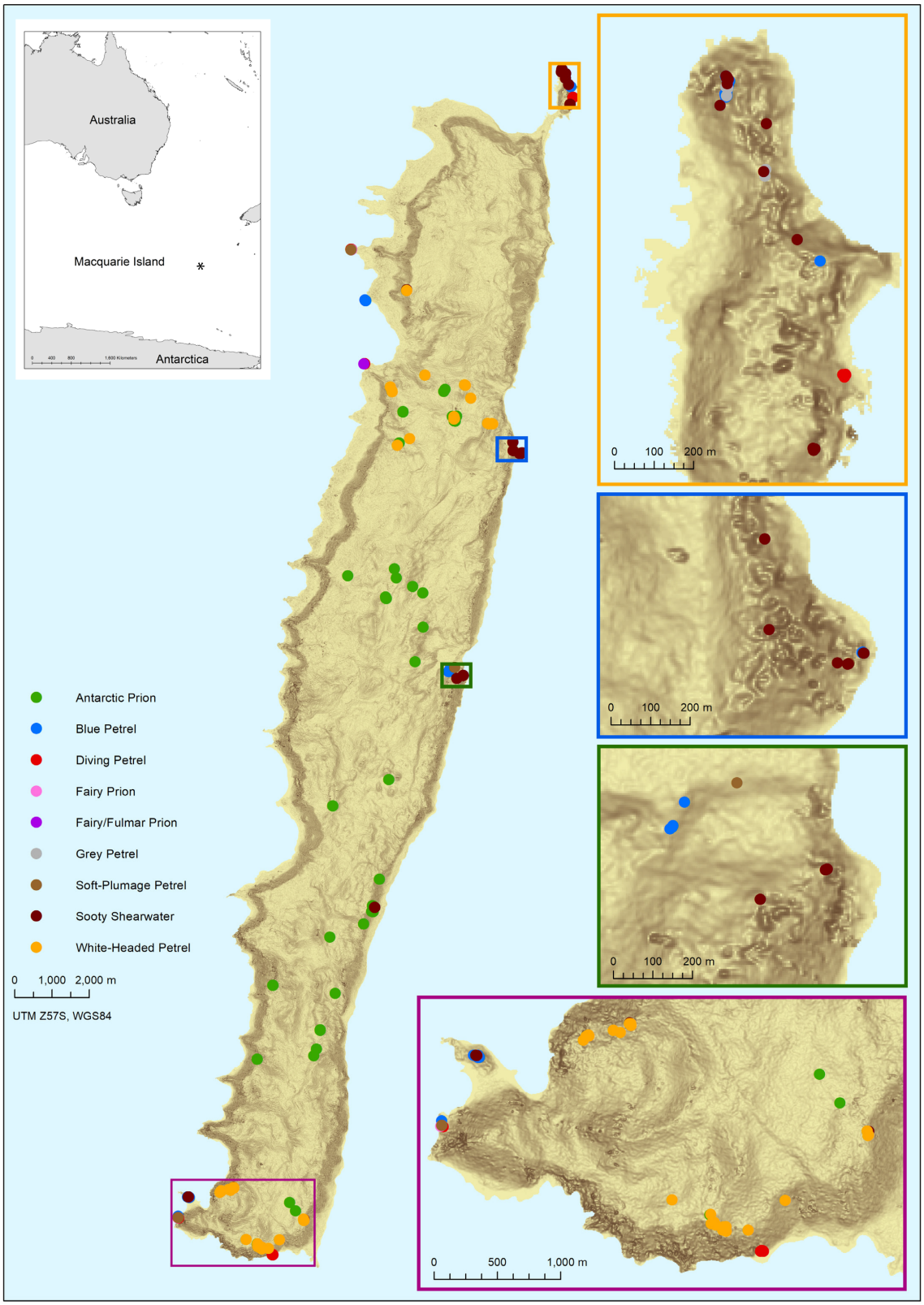


**FIGURE 1** Sampling locations on Macquarie Island in 2017–2018 where burrowing petrel species identification was confirmed using DNA from scats and feathers. NB: we could not distinguish between fulmar and fairy prion DNA in two samples

## 2 | CASE STUDY

Petrels and shearwaters are one of the most threatened groups of species globally (Dias et al., 2019). They face multiple ongoing threats including predation pressure from invasive species, fishery bycatch, and changing environmental conditions (Dias et al., 2019; Rodríguez et al., 2019). As a result, 42% are listed as threatened and 52% suffering population declines (IUCN, 2020; Rodríguez et al., 2019). Understanding how species diversity varies in response to threats or conservation actions is important for monitoring programs and land managers. However, petrels are particularly difficult to detect because they are only present seasonally, only active at colonies at night, are hidden in underground burrows by day, and their colonies are fragile hampering survey access (Rodríguez et al., 2019). We aimed to test the efficacy of low-impact DNA analysis using high-throughput sequencing to identify burrowing petrel species in a changing island ecosystem, recently released from invasive species predation pressure.

At least nine species of burrowing petrels have been recorded breeding on Macquarie Island and its offshore rock-stacks (Table 1, Brothers, 1984; Schulz, Robinson, & Gales, 2005; DPIW, 2007). Burrowing petrels were more abundant and widespread prior to the arrival of multiple invasive species (Brothers, 1984). Only three species, Antarctic prions (*Pachyptila desolata*), sooty shearwaters (*Ardenna grisea*) and white-headed petrels (*Pterodroma lessonii*) commonly bred on the main island in the presence of wekas (*Gallirallus australis scotti*), cats (*Felis catus*), black rats (*Rattus rattus*), house mice (*Mus musculus*), and European rabbits (*Oryctolagus cuniculus*), while blue petrels (*Halobaena caerulea*), diving-petrels (*Pelecanoides spp.*), fairy prions (*Pachyptila turtur*), and Wilson's storm petrels (*Oceanites oceanicus*) were restricted to offshore stacks free of invasives (Brothers, 1984; Brothers & Ledingham, 2008). Wekas were eradicated in 1989 and cats in 2000 (Springer, 2016). Following cat eradication, grey petrels (*Procellaria cinerea*) and soft-plumaged petrels (*Pterodroma mollis*) were resighted breeding on the main island (DPIW, 2007; Schulz et al., 2005). In 2014, the remaining invasive vertebrates (rats, mice, and rabbits) were eradicated (Springer, 2016). In 2017, extensive whole island field surveys were undertaken to identify the presence and distribution of all burrowing petrel species on Macquarie Island.

Scat and discarded feather samples were collected from across Macquarie Island (222 scat and 108 feather samples) between November 2017 and November 2018. Samples were collected within seabird colonies during ground searches and GPS coordinates were taken for each sample collected (see Appendix S1 for further sampling details). To evaluate which scat samples provided sufficient bird DNA, the freshness of scat samples was recorded as either "old" when a sample was dry and compacted, or "recent" when a sample was wet as this can affect the amplification success (McInnes et al., 2017). The pigment of a subset of samples was also recorded, as DNA-based diet studies have shown that all white samples contain minimal prey DNA (Thalinger, Oehm, Obwexer, & Traugott, 2017).

Details of the molecular methods are provided in Appendix S1. Briefly, DNA was extracted from all samples and two short mitochondrial markers (COI and 16S) were amplified using custom-designed burrowing petrel

**TABLE 1** Burrowing petrel species detected using DNA analysis of scat and feather samples on Macquarie Island from November 2017 to November 2019

| | | | | Scat samples | | | |
|---|---|---|---|---|---|---|---|
| | Common name | Species | Feather samples | Recent | Old | Not recorded | Total samples |
| Species match | Antarctic prion | *Pachyptila desolata* | 30 | 10 | 21 | 10 | 71 |
| | Blue petrel | *Halobaena caerulea* | 44 | 5 | 11 | 1 | 61 |
| | Fairy prion | *Pachyptila turtur* | | 1 | 1 | | 2 |
| | Grey petrel | *Procellaria cinerea* | | 2 | 3 | 1 | 6 |
| | Soft-plumage petrel | *Pterodroma mollis* | 5 | | 1 | | 6 |
| | Sooty shearwater | *Ardenna grisea* | | 21 | 24 | 1 | 46 |
| | White-headed petrel | *Pterodroma lessonii* | 3 | 18 | 33 | 4 | 58 |
| Species unconfirmed | Diving petrel | *Pelecanoides urinatrix/ georgicus* | 8 | 14 | 5 | 1 | 28 |
| | Fulmar prion | *Pachyptila crassirostris* | | 2 | | | 2 |

primers. Amplicons were pooled and sequenced on an Ilumina Miseq and compared to reference databases to provide species-level identifications. To assign species, maximum likelihood trees were constructed using MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) with representative species sequences from field samples and reference sequences from the Barcode of Life Database (BOLD). Using these data, we identify species presence and mapped the locations where species were detected with DNA metabarcoding.

## 3 | DNA VIABILITY OF DEGRADED SAMPLES

Avian DNA was successfully amplified in 303 samples, comprised of 209 scat samples (94% success) and 94 feather samples (87% success). There was no significant difference in the amplification success of feathers and scats for either the CO1 marker ($X^2 = 0.331$, $p > .05$), or the 16S marker ($X^2 = 0.817$, $p > .05$). Amplified bird DNA was obtained from 91% of "old" scat samples ($n = 123$) and 100% of "recent" samples ($n = 79$). For the subset of samples where color 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) were not burrowing petrel species (e.g., kelp gull *Larus dominicanus*) and were excluded from further analysis, giving a total of 280 burrowing petrel DNA samples. Although there was no difference in amplification success of scat and feather DNA, there was a difference in availability of each sample type for some species (Table 1).

## 4 | SPECIES DETECTION

We detected DNA from eight of the nine burrowing petrel species previously recorded breeding on Macquarie Island in the last 40 years (Table 1), including fairy prions and diving petrels which are rarely detected (Brothers, 1984; Brothers & Bone, 2008). At sites where surveys were restricted to daytime ground-searches (e.g., rock-stacks) we detected additional species from DNA samples such as soft-plumaged petrel and fairy prions that were not otherwise recorded. Nocturnal spotlight surveys detected the same or additional species to those detected via DNA sampling. This highlights that DNA monitoring is a useful addition where field surveys are constrained, for example, limited to daylight or when field personnel are inexperienced in spotlight surveys.

## 5 | SPECIES IDENTIFICATION AND TAXONOMY

Our diving-petrel samples had a 100% match with online reference sequences from South Georgia diving-petrels (*Pelecanoides georgicus*). As there were only two base pairs difference from common diving-petrel (*P. urinatrix*), a separate gene region (Cytochrome B) was used to confirm this genetic result. This finding was of interest because these birds had been identified from our field surveys as common diving-petrels based upon morphology, acoustics, phenology and habitat preference. Since the taxonomy of the diving petrels and integration with molecular data is an area of active research, we have taken the approach of assigning these only to genus during this study. We aim to investigate further as new genetic information becomes available.

For prions, two samples matched fairy prion reference sequences, but a further two were intermediate between fulmar prion (*Pachyptila crassirostris*) and fairy prion reference sequences (Figure 2) and thus the presence of fulmar prions could not be ruled out.

Our study did not set out to investigate species provenance and population structure, yet some interesting outcomes emerged. Two sequences generated from white-headed petrel samples were either an exact match, or one base pair different, to a reference sequence from an Auckland Islands specimen. However, the Macquarie Island sequences were only a 97% match with white-headed petrel reference sequences from the Antipodes Islands (Figure 2). The genetic difference detected between white-headed petrels from Macquarie Island and the Antipodes Islands matches previously reported morphological differences (Wood et al., 2017) and highlights the high level of intra-species variability in this species. The South Georgia diving-petrel samples mentioned above also belonged to a distinctive mitochondrial lineage (>7% COI intraspecific divergence) that has previously been reported in the Atlantic, but not in Pacific or Indian sectors of the Southern Ocean (Figure 2). The sequences from Antarctic prions, sooty shearwaters and grey petrels matched respective reference sequences online and did not vary in the Macquarie Island samples; however, faster evolving gene regions could be used to further investigate these species.

## 6 | BENEFITS OF MOLECULAR METHODS IN CONSERVATION PROGRAMS

The use of molecular methods assisted in the detection of new species arrivals and new sites on a 12,900 ha island

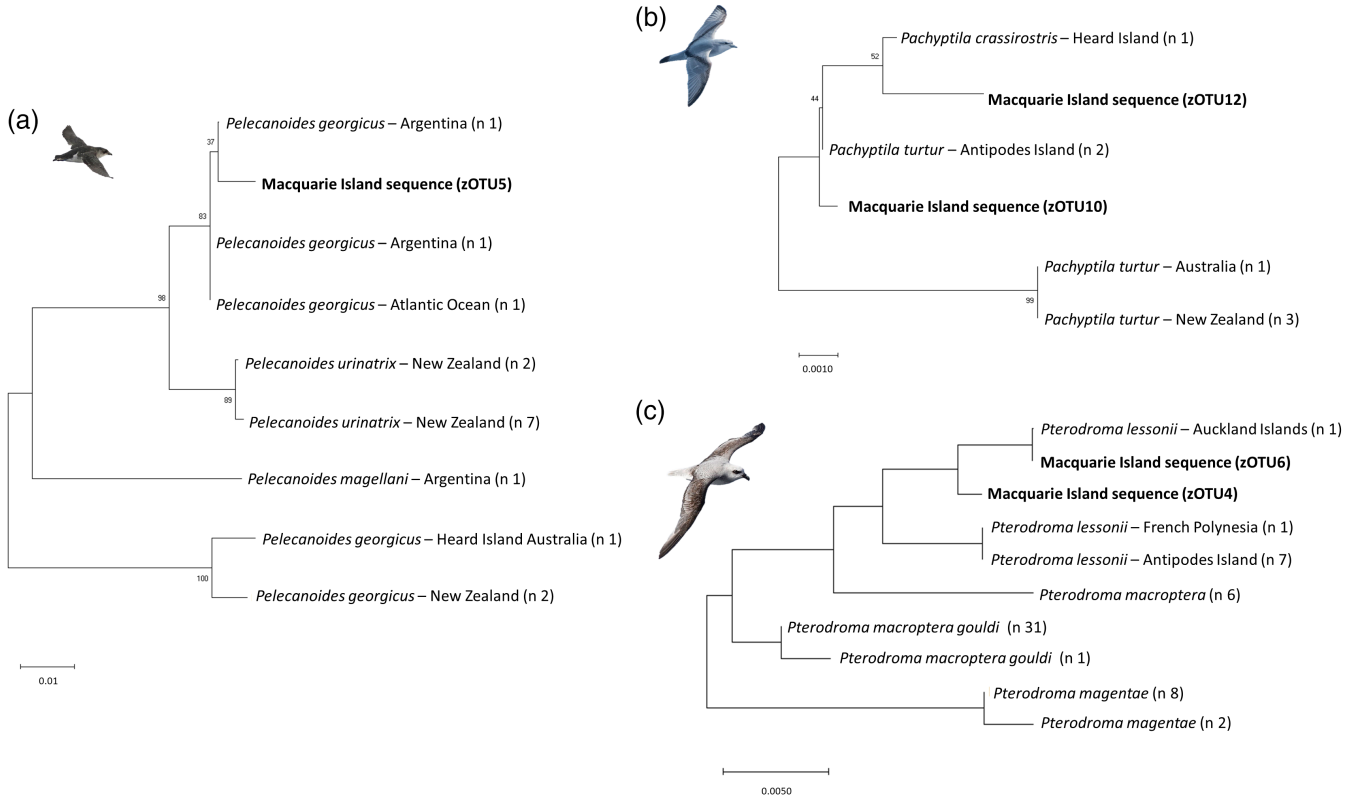


**FIGURE 2** Trees generated from CO1 sequences obtained from (a) diving petrels (*Pelacanoides*), (b) fulmar/fairy prions (*Pachypilta turtur/crassirostris*), and (c) white-headed petrels (*Pterodroma lessonii*). Trees were created using the maximum likelihood method with the longest available reference sequences for close matches (629, 649, and 426 base pairs, respectively), along with the Macquarie Island sequences (bold) from the current study (142 base pairs). Branch lengths are measured in the number of substitutions per site. Sample sizes reflect the number of reference sequences available on BOLD and Genbank databases

that is undergoing rapid change in response to vertebrate pest eradication, and provided preliminary insights into provenance of recolonizing populations. Both feathers and scats provided high quality DNA for species identification. Importantly our work called into question established field identifications for one species. This study also highlighted potential intra-specific genetic differences between Macquarie Island populations and voucher specimens previously collected on other sub-Antarctic islands. Examining the population genetics of these species highlights the importance of range-wide conservation and can provide insights into potential source populations that may be driving population increases in response to vertebrate pest eradication. Notably, these population-level differences would never be detected through traditional field surveys. Our results highlight the underutilized potential of DNA metabarcoding as a valuable tool to complement field surveys of seabirds.

## ACKNOWLEDGMENTS

This research was supported by funding from the Australian Government's National Environmental Science Programme through the Threatened Species Recovery Hub and the Australian Antarctic Science program (AAS 4305). Sample collections were carried out under the Australian Antarctic Science project 4305, University of Queensland Permit AE 29713, Scientific Collection permit FA 17305, and in collaboration with the Department of Primary Industries, Parks, Water and Environment. Thanks to R. Alderman for species advice, and P. Pascoe and T. Travers for assistance collecting samples.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

**Julie C. McInnes**, **Justine D. Shaw**, and **Bruce E. Deagle**: Conceptualized and designed the project. **Julie C. McInnes** and **Jeremy P. Bird**: Carried out the fieldwork. **Julie C. McInnes** and **Andrea Polanowski**: Carried out the laboratory work. All authors contributed to the manuscript.

## DATA AVAILABILITY STATEMENT

All data will be available through the Australian Antarctic Data Centre doi:10.26179/5bkj-yg59.

## ETHICS STATEMENT

This project was carried out under the University of Queensland Animal Ethics Permit AE 29713.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** McInnes JC, Bird JP, Deagle BE, Polanowski AM, Shaw JD. Using DNA metabarcoding to detect burrowing seabirds in a remote landscape. *Conservation Science and Practice*. 2021;3:e439. https://doi.org/10.1111/csp2.439