

Supplementary Information for

Subsistence practices, past biodiversity, and anthropogenic impacts revealed by New Zealand-wide ancient DNA survey

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Supplementary Information Text

1. Contamination and introduced species

To monitor laboratory contamination, at least two negative controls were included in each batch of sample preparations (see Table S5). Extraction and PCR-blanks represent no-template controls for the extraction and PCR setup protocols, respectively. Grinding blanks were prepared by running the ball mill with 15 mL of ultrapure water in the grinding pod and concentrating the water to 500 μ L using an Amicon®Ultra-4 Centrifugal Filter (Millipore).

In the controls we identify a low level of background contamination from the three well-known contaminants *Homo sapiens*, Phasianinae (presumably *Gallus gallus*) and *Sus* (1) (see Table S7). Accordingly, reads assigned to these three species in the bulk-bone samples were excluded from downstream analyses. In addition, we identified one critical contamination from red kangaroo (*Marcopus rufus*) in a single sample from Cobden cave. As ancient DNA from this species has been studied previously in our laboratory, and since only 58 contaminant reads are identified out of 37,386 (0.16%), it is likely that the DNA represents low-level background contamination. Furthermore, red kangaroo has never been present in New Zealand prior to European arrival, and was not amongst the species introduced by Europeans (e.g. wallabies). To investigate whether contamination occurred at the bone grinding, DNA extraction, or PCR amplification stages, the remaining bones from Cobden cave were ground and extracted (Cob_A_NZ4), two new extracts were made from the original bone powder (Cob_B_NZ4, Cob_C_NZ4), and a second amplification was carried out from the original extract (Cob_NZ1). As none of these yielded any DNA from *M. rufus*, and since no DNA of *M. rufus* was amplified in any of our blanks or other test samples, we conclude that this is a product of a single contamination incidence. The *M. rufus* DNA is most likely a result of a contaminated tagged primer or a combination of post-amplification contamination and tag-jumping. Furthermore, most samples were prepared in batches from very different bulk-bone projects (e.g., geographically distinct sites) to better detect cross contamination between DNA extracts. Reassuringly, we do not detect species endemic to New Zealand in data from other projects or vice versa.

Additionally, a handful of introduced species are identified among the different samples analyzed. These fall into two categories: (i) common contaminants and (ii) other introduced species. The former group consists of the domesticates cattle (*Bos*), sheep (*Ovis*), goat (*Capra*), and cat (*Felis sylvestris*), and are by far the most commonly identified introduced species in the dataset, detected at 12 midden sites, seven paleontological sites and one mixed site. However, as these are well-known laboratory contaminants, it is challenging to determine whether this DNA is endogenous. Furthermore, sheep DNA has previously been shown to leak into moa deposits at the Hukanui pool site (2) and, like sheep, both goat and cattle produce large volumes of urine and fecal material, that could leak into both midden and cave deposits. Moreover, the notion that cattle and sheep DNA could stem from background contamination or DNA leaching is reinforced by the fact that these species, along with human and pig, are the only taxa identified in the samples from Lake Poukawa, where no endogenous DNA could be amplified. However, it is also possible that early historic-period deposition by Māori or Europeans at some sites can have contaminated bulk bone samples with historic introduced species. The cultural layers derived from pre-contact Māori activity typically represent a thin horizon and light sediment layer perturbation could cause overlying post-European layers to contaminate pre-historic layers with remains from introduced species. Lastly, while the detection of dog (*Canis lupus familiaris*) is expected in archaeological sites, we cannot rule out the possibility that the dog DNA could stem from laboratory contamination as dog is a common contaminant.

Taxa from the second group, on the other hand, most likely represent endogenous DNA from bones that have made their way into the deposits from the surface by post-depositional reworking of the sediment or post-European deposition. This group contains common brushtail possum (*Trichosurus vulpecula*), rabbit (*Oryctolagus cuniculus*), hare (*Lepus europaeus*), ship rat (*Rattus rattus*), and European goldfinch (*Carduelis carduelis*), and these species are detected much less frequently than group 1 (six paleontological and no

archaeological sites). All of these species are found in New Zealand today and the detection of these corresponds well to the morphological record, where modern day species are identified occasionally in the upper layers of bone deposits (3-6).

2. Contributions and limitations of morphology and BBM

Both morphological analysis and BBM are challenged by similar inherent limitations, and each methodology cannot easily stand alone. In both methods, failure to detect a taxon does not necessarily translate to absence from the deposit, but can be explained by a number of factors: In BBM, the ability to detect species may be affected by primer specificity, variable DNA preservation and the availability of relevant reference sequences, whereas, for morphological identification, taphonomic factors and the level of taxonomic expertise available could cause species to be missed, or incorrectly assigned. In our data, this is illustrated by the detection of species that are frequently missed by morphological approaches, such as whales, frogs and passerine birds, along with the absence of commonly detected species such as the New Zealand owl nightjar, which does not have a relevant reference sequence available. Furthermore, owing to the different number of bones sampled for each approach (hundreds for BBM and thousands for morphology), the number of taxa identified with BBM is generally lower than that of morphology. Hence, here, BBM offers a conservative estimate of past biodiversity, providing a lower threshold of the past species richness in New Zealand which can be built on in future research. In particular, the emphasis on spatial patterns in the current study, warrants an examination of undiagnostic bones from a single site with a long depositional history, which could expand on the data presented here by improving our understanding of the exact temporal dynamics of biodiversity turnover in New Zealand. Ideally, BBM will become more widely applied in palaeontological and archaeological practice (51), and seen as a powerful complement to traditional analyses.

3. Palimpsest deposits.

The midden deposits investigated here are examples of cumulative palimpsests (53), in which the lack of fine stratigraphic differentiation along with the mixing of materials prevent temporal precision. Midden deposits are a result of subsistence practices which are formed when leftover bones are discarded. This is a complex process affected not only by the hunting episode itself, but by transportation issues, butchering methods and cooking practices. Additionally, post-depositional factors such as reworking of sediments by rodents and commensals along with taphonomic processes variably affecting different parts of the assemblage further blurs the original pattern. As such, individual hunting episodes are not discernible within the midden sequence, rather the deposits provide information on subsistence practices over longer time spans. However, this also highlights the advantage of cumulative palimpsest midden assemblages: with the repetition of similar hunting events at the same location over long time spans, large deposits are formed which are more easily analyzed and provide strong evidence for general trends at one location.

4. Materials and Methods

4.1 Subsampling and extraction. Bone fragments were subsampled into bulk bone pools of approximately 100 (batch: NZ1) or 50 bones each (batches: NZ2-5; *SI Appendix*, Table S9) of roughly equal size. For larger bones, a small fragment was broken off and added to the subsample. Depending on the size of each bulk bone sample, between one and five bulk bone pools were subsampled for each site. All subsamples were ground using a Retsch PM200 Planetary Ball Mill at 400 rpm until pulverized. Extractions were carried out with approximately 100 mg of bone powder dissolved in digestion buffer (0.25 mg Proteinase K + 1 mL 0.5 M EDTA) and incubated with rotation over night at 55°C. Next, samples were centrifuged, and the supernatant was concentrated to 50 µL in a MWCO 30,000 Vivaspin 500

column (Sigma-Aldrich). Lastly, the DNA was purified using the MinElute PCR Purification Kit (Qiagen) following the manufacturer's instructions, except for the use of a modified binding buffer optimized for ancient DNA(46) (40% Isopropanol, 0.05% μ L Tween 20, 90 nm NaAc and 5M GuanHydCh in Ultrapure water).

4.2 Barcoding. All barcoding primers were fused to Illumina sequencing adaptors and tagged by a unique combination of 6-8 bp indexes on each primer. For metabarcoding assays, 25 μ L PCR reactions were setup with 1 μ L of template DNA, 1X buffer (ThermoFisher), 2mM $MgCl_2$ 0.25mM dNTPs, 1U AmpliTaq Gold[®] DNA Polymerase, 0.6 μ L 5X SYBR green, 0.4 mg/ml BSA and 0.4 μ M of primers. DNA was amplified on an Applied Biosystems StepOnePlus[™] Real-Time PCR System with the following cycling conditions: an initial denaturation step at 95°C for 10 min, followed by 50 cycles of 95°C for 30 sec, 57/54°C for 30 sec (*SI Appendix*, Table S2) and 72°C for 45 sec; and a final elongation step of 72°C for 10 min. For the kākāpō specific barcoding assay, PCR reactions were setup as described above with one of the primer sets described in Bergner et al. (Table S2). This region was chosen because it had the fewest low complexity strings and length variable regions of the 4 assays described by Bergner et al. PCR reactions were setup in four replicates for each extract under the following cycling conditions: an initial denaturation step at 95°C for 5 min, followed by 50 cycles of 95°C for 30 sec, 52/58°C for 30 sec (*SI Appendix*, Table S2) and 72°C for 2 min; and a final elongation step of 72°C for 10 min. After PCR amplification, all reactions (metabarcoding and kākāpō specific assays) were pooled in equimolar concentrations based on end point fluorescence intensity, and LabChip GX Touch HT (PerkinElmer) quantifications. Lastly, amplicons were sequenced unidirectionally on the Illumina MiSeq platform using the 300-cycle MiSeq Reagent Nano Kit v2 (Illumina) with custom sequencing primers.

4.3 Sequence analysis. Sequences were filtered using a custom-made pipeline based on OBITools(56) (<http://www.grenoble.prabi.fr/trac/OBITools>; *SI Appendix*, Table S10). Firstly, samples were demultiplexed and adapter and primer sequences were removed using ngsfilter, only retaining reads with a 100% match to both tags and primers. Next, samples were dereplicated (obiuniq), discarding reads shorter than 80 bp, or reads represented by fewer than 5 replicates (obigrep -l 80 -p "count>=5"). To account for PCR and sequencing errors, the data was denoised using the obiclean program (obiclean -r 0.2 -d 2 -H) and Sumacust, collapsing clusters at 95% and 93% with abundance thresholds of 50% and 1%, respectively, in a sample-wise manner. Lastly, chimeric sequences were removed using vsearch (vsearch -uchime_denovo)(57).

4.4 Taxonomic assignments. Raw taxonomic assignments were achieved using the script blast_getLCA.py (https://github.com/frederikseersholm/blast_getLCA) where reads are assigned to the taxonomic node of the lowest common ancestor of the best hits to the database based on percent similarity. Briefly, filtered and denoised sequences were queried against the NCBI nt database (<ftp://ftp.ncbi.nlm.nih.gov/blast/db/nt.gz>), downloaded 31st of August 2017(58)) and a local database of the reference sequences for *Leiopelma markhami*, *Nestor meridionalis*, *Circus teauteensis* and *Aquila moorei* using the megablast algorithm (59). Next, blast-files were parsed using the script blast_getLCA.py assigning each read to the taxonomic node of the lowest common ancestor of the best hits to the database based on percent similarity. To ensure that species level identifications were only called based on high confidence alignments, species level assignments were made only on hits with above 98% similarity to the reference, with assignments between 95% and 98% called at the genus level (7). Lastly, after this raw taxonomic assignment, each taxonomic node was examined and correlated to records of species present in New Zealand and the availability of reference sequences of similar species known from New Zealand (NCBI genbank). In cases of lower taxonomic nodes only represented by a single New Zealand species, the assignment was upgraded to species level, e.g., *Falco* was reassigned to *Falco novaeseelandiae* as this is the

only falcon present in New Zealand. Likewise, in cases of species level assignments where similar species within the same genus from New Zealand were absent from the reference database, the assignment was downgraded to genus level. e.g., *Cyanoramphus novaezelandiae* was reassigned to *Cyanoramphus*, as other *Cyanoramphus* species from New Zealand are absent from the database.

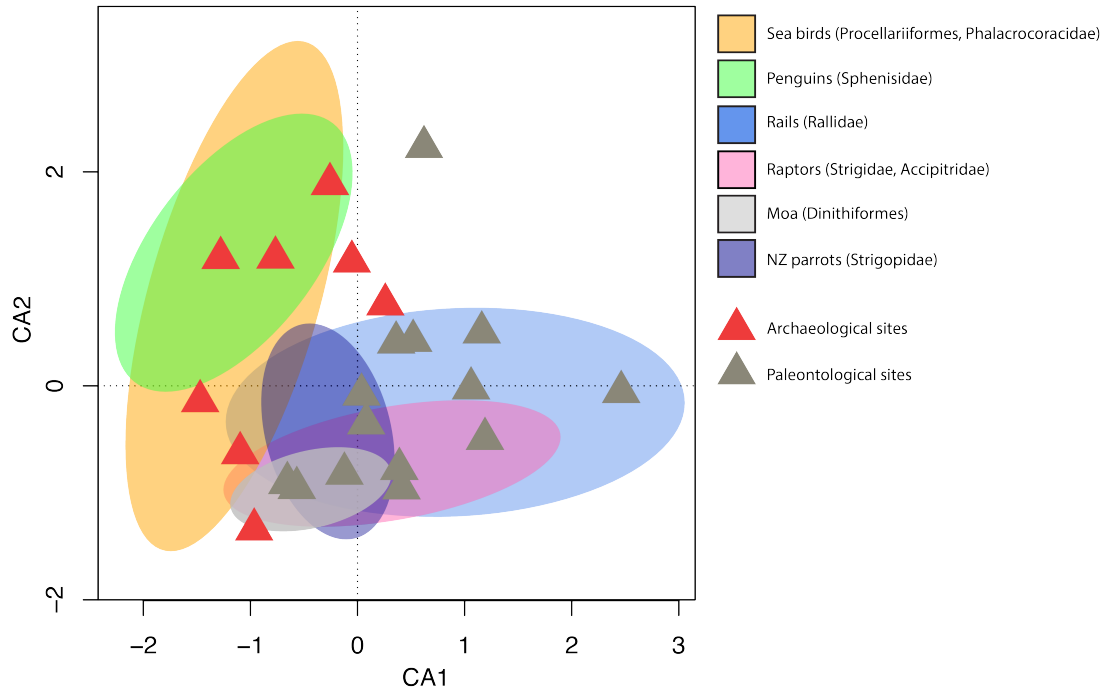


Fig. S1. Avifauna structure. Coordination analysis of bird species composition from paleontological (gray triangles) and archaeological sites (red triangles) based on presence/absence. Sites represented by three or fewer bird taxa have been omitted.

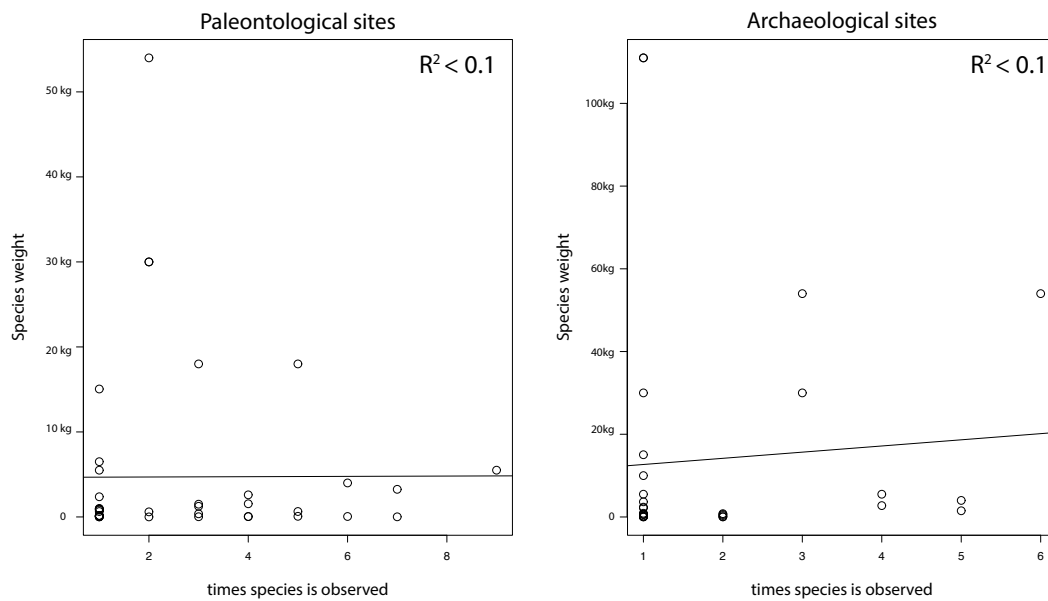


Fig. S2. Bird size and abundance comparisons. Only bird taxa identified at species or subspecies level were considered. Estimates of bird weights are the averages of male and female weights from <http://nzbirdsonline.org.nz/>.

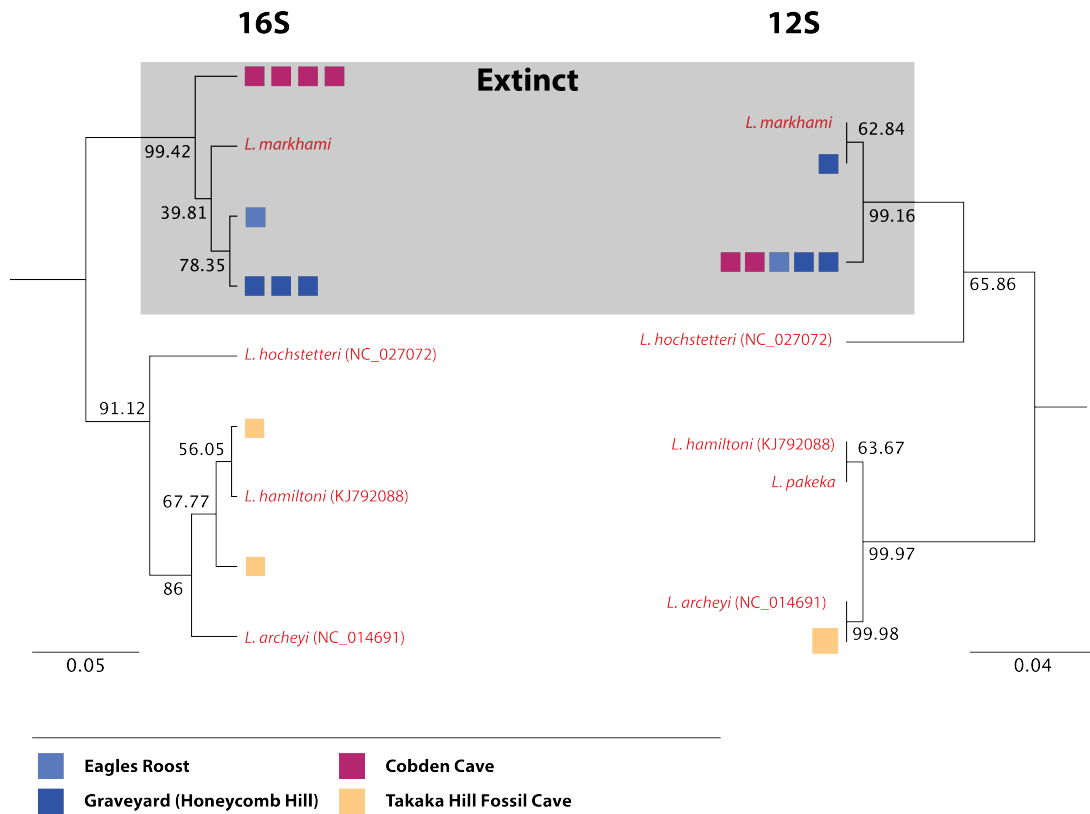


Fig. S3. *Leiopelma* spp. diversity. UPGMA consensus tree with 10,000 bootstraps of 12S and 16S barcodes assigned within Leiopelmatidae. *L. markhami* reference sequences were generated for this study while the *L. pakeka* 12S sequence was provided by Luke Easton and Nicolas Rawlence.

Archaeological sites ▲

- AM, Awamoā
- CC, Cooks cove
- Fyf, Fyffe site
- Hoh, Hohouponamu
- Kap, Kapiti Coast
- Kaw, Kawatiri
- KK, Kahukura
- Lon, Long Beach
- MonC, Moncks Cave
- Oma, Omaio
- R27, Main Road 27
- Rmo, Moabone Point Cave
- SC, St Clair
- She, Shepherds Creek
- SP, Shag Point
- Tim, Dashing Rocks
- TN, Tokanui
- Tum, Tumbledown Bay
- Wai, Wairau Bar
- Wak, Wakanui
- Wat, Watsons Beach
- Wha, Whalers Bay Cave
- Wm, Waimataitai

**Archaeological/
palaeontological sites ▲**

- Dur, D'Urville Island
- Tok, Tokerau Beach

Palaeological sites ▲

- Ard, Ardenest
- Cob, Cobden Cave
- EaR, Eagles Roost
- Fal, Falcon site
- FF, Finsch's Folly
- Gor, Gordons Valley Station
- Gou, Goulands Downs Cave
- GY, Graveyard
- Kid, Kids Cave
- MarC, Martinborough Cave #1
- MtC, Owl Site, Annandale Stn
- Pou, Poukawa XII
- Pre, Predator Cave
- Tak, Takaka Fossil cave
- TeW, Te Waka #1

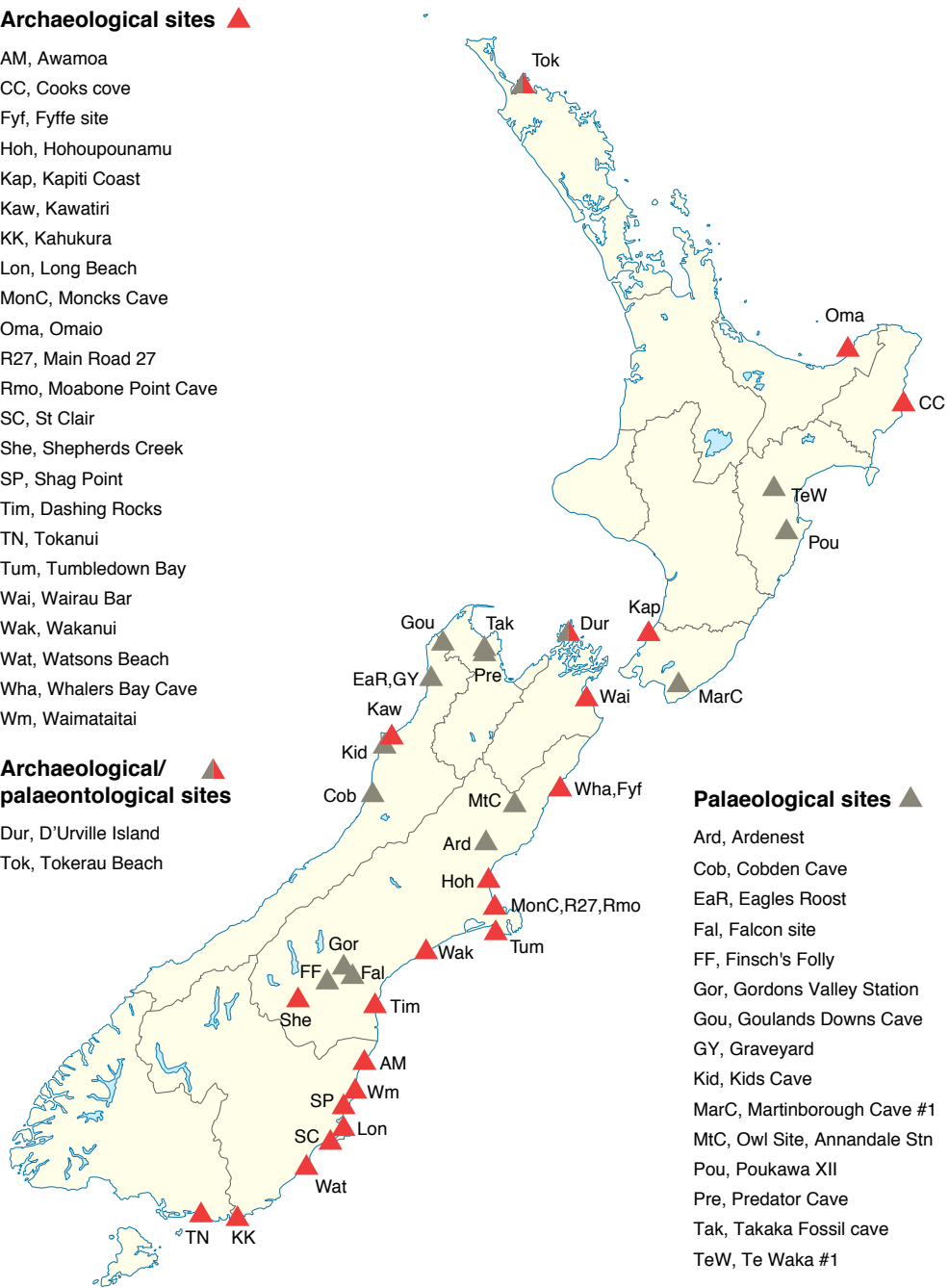


Fig. S4. Site locations.

- ② Bulk bone sites where each species was detected
- Time range for extinct birds
- Time range for extinct herpetofauna

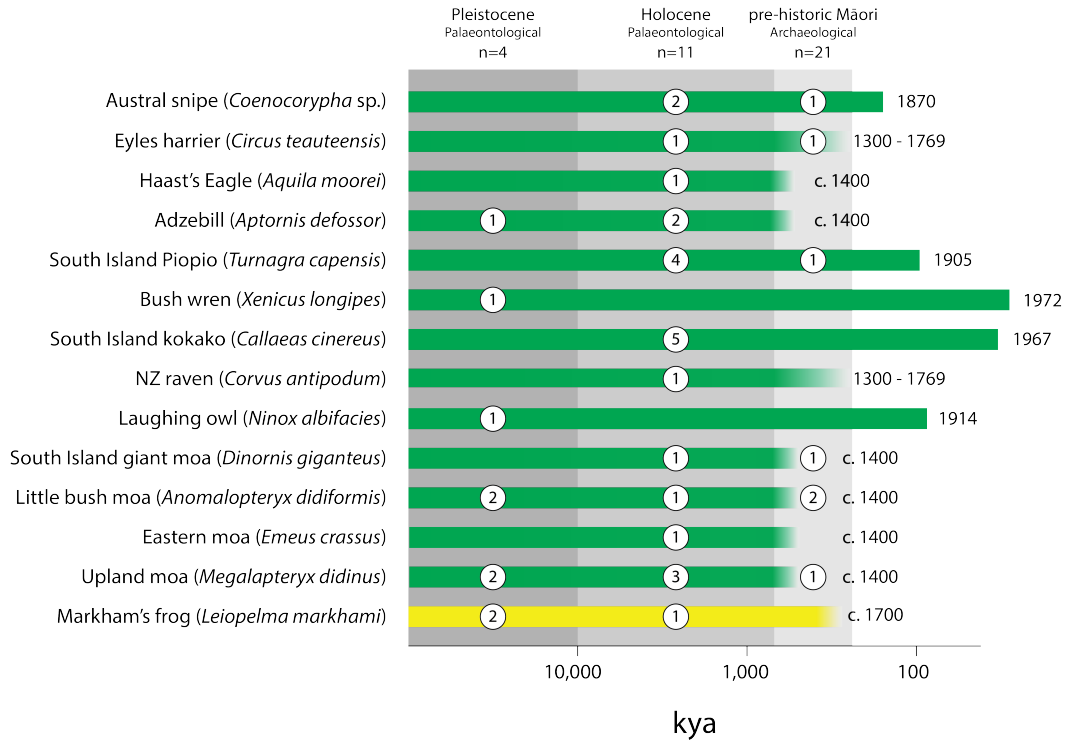


Fig. S5. Extinction times. Extinction times (AD) for extinct species detected in the current study, with information on the number of assemblages in which each species was detected. Sites were binned in Pleistocene, Holocene and pre-historic Māori assemblages (see table S1).

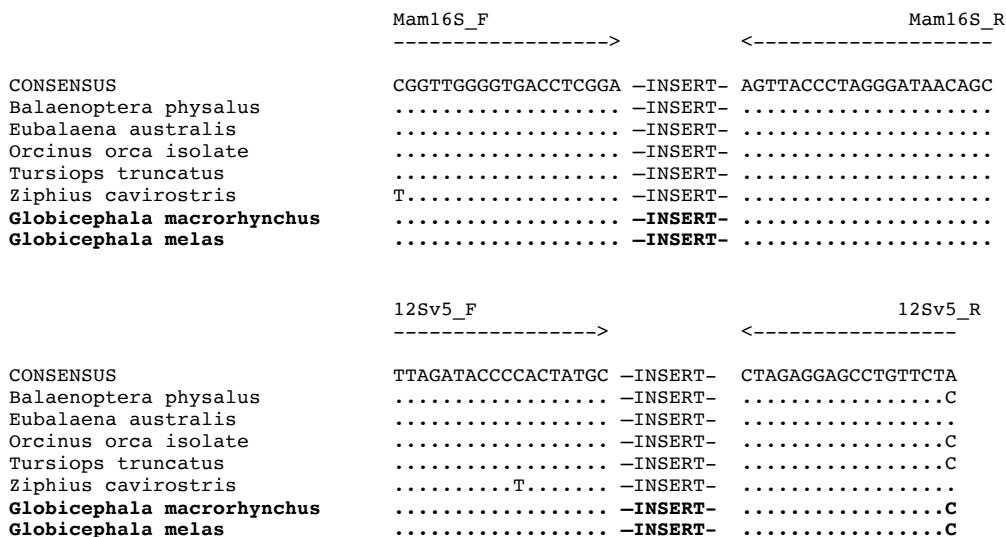


Fig. S6. In silico evaluation of pilot whale primer binding. Primer binding sites for the five whale species detected in this study, along with the two species of pilot whale. For the 16S primer set, the pilot whales have no mismatches to the primer sequences, whereas, for the 12S primer set, there is a single 5' mismatch to the reverse primer unlikely to impact on PCR efficacy.

Table S1. Site descriptions.

Short ID	Site name	Location	Collection type	Island	Collected from	Time period	Age	Reference
CC	Cooks Cove	Tolaga Bay	archaeo	North	University of Otago	Māori	AD 1300-1800	Walter et al. 2011 (8)
Kap	Waikanae	Kapiti Coast	archaeo	North	University of Otago	Māori	~ AD 1300-1800	NZAA #: R26/486
Oma	Omaio		archaeo	North	University of Otago	Late Māori	AD 1700-1800	Walter 2010 (10)
Lon	Long Beach	Otago	archaeo	South	University of Otago	Late Māori	AD 1550-1700	Leach et al. 1981 (11)
R27	Main Road 27	Redcliffs	archaeo	South	University of Otago	Early Māori	AD 1350-1400	Jacomb 2009 (12)
Rmo	Moabone Point Cave	Redcliffs	archaeo	South	Canterbury Museum	Early Māori	AD 1300-1400	Trotter 1975 (13)
MonC	Moncks Cave	Redcliffs	archaeo	South	Canterbury Museum	Early Māori	AD 1350-1450	Trotter 1975 (13)
Wai	Wairau Bar	Marlborough	archaeo	South	University of Otago, Canterbury Museum	Early Māori	AD 1285-1300	Higham et al. 1999 (14)
TN	Tokenui		archaeo	South	University of Otago	Early Māori	~AD 1300-1400	Jacomb 2011 (15)
KK	Kahukura		archaeo	South	University of Otago	Early Māori	AD 1450-1500	Cunliffe et al. 2016 (16)
Kaw	Kawatiri	Buller	archaeo	South	University of Otago	Early Māori	AD 1300-1350	Walter et al 2011 (17)
Wm	Waimataitai	North Otago	archaeo	South	Canterbury Museum	Early Māori	Before ~AD 1400	Trotter 1955 (18)
Fyf	Fyffe Site	Kaikoura	archaeo	South	Canterbury Museum	Early Māori	~ AD 1300	McFadgen 1987 (19)
Wak	Wakanui		archaeo	South	Canterbury Museum	Early Māori	~ AD 1300-1500	Trotter 1975 (20)
SP	Shag Point	North Otago	archaeo	South	Canterbury Museum	Māori	AD 1400-1700	Weisler 2000 (21)
Tum	Tumbledown Bay	Banks Peninsula	archaeo	South	Canterbury Museum	Māori	~ AD 1300-1700	Mason 1963a (23)
Tim	Dashing Rocks	Timaru	archaeo	South	Canterbury Museum	Māori	~ AD 1300-1700	Mason 1963b (24)
AM	Awamoa	North Otago	archaeo	South	University of Otago	Early Māori	AD 1300-1450	Harrowfield 2013 (25)
Wha	Whalers Bay Cave	Kaikoura	archaeo	South	Canterbury Museum	Māori	~ AD 1300-1700	Burrage 1968
Wat	Watsons Beach		archaeo	South	University of Otago	Māori	~AD 1400-1600	Darmody 2002 (27)
SC	St Clair	Otago	archaeo	South	University of Otago	Early Māori	~AD 1300-1500	Teviotdale 1932 (28)
Tok	Tokerau Beach	Northland	archaeo	North	Canterbury Museum	Māori/Holocene	Late Holocene	Fredericksen 1996 (9)
Dur	D'Urville Island	Marlborough Sounds	archaeo/paleo	South	Te Papa	Māori/Holocene	Late Holocene	
MarC	Martinborough Cave #1	Ruakokopatuna	paleo	North	University of Otago	Holocene	~1,500 BP	Worthy 1987 (1)
Cob	Cobden Cave	Greymouth	paleo	South	Te Papa	Holocene	3,700 – 650 BP	Worthy et al. 2002 (31)
Kid	Kids Cave	West Coast	paleo	South	Te Papa	Pleistocene	22,000-15,000 BP	Worthy et al. 2006 (3)
Tak	Takaka Fossil cave	Takaka hill	paleo	South	Te Papa	Holocene	12,000-1,600 BP	Worthy et al. 2003 (4)
Ard	Ardenest	Waikari Valley	paleo	South	Te Papa	Holocene	3,000 BP - present	Worthy et al. 1996 (32)
MtC	Owl Site, Annandale Stn	Mt Cookson	paleo	South	Te Papa	Holocene	Late Holocene	Worthy et al. 1995 (33)
Pou	Poukawa XII	Hawkes Bay	paleo	North	Te Papa	Holocene	7,000 BP – AD 1700	Worthy 2004 (34)
TeW	Te Waka #1	Hawkes Bay	paleo	North	Te Papa	Pleistocene	22,600 BP - present	Worthy 2002 (5)
Gor	Gordons Valley Station	South Canterbury	paleo	South	Te Papa	Holocene	~10,000-5,000 BP	Worthy 1997 (6)
Pre	Predator Cave	Takaka Hill	paleo	South	Te Papa	Holocene	10,000 - present	Worthy 1994 (35)
Fal	Falcon site	Braeburn, South Canterbury	paleo	South	Te Papa	Holocene	~10,000-5,000 BP	Worthy 1997 (6)
Gou	Goulands Downs Cave	Nelson	paleo	South	Te Papa	Holocene	Holocene	Worthy 2001 (36)
GY	Graveyard	Honeycomb Hill	paleo	South	Te Papa	Pleistocene	20,000-11,000 BP	Worthy 1989 (37)
EaR	Eagles Roost	Honeycomb Hill	paleo	South	University of Otago	Pleistocene	14,000-11,000 BP	Worthy 1989 (37)
FF	Finsch's Folly	South Canterbury	paleo	South	Grealy et al. 2015(2)	Holocene	1600 – 1150 BP	Grealy et al. 2015 (38)

Table S2. Primers.

	<i>Forward Primer</i>	<i>Reverse Primer</i>	<i>Annealing Temp</i>	<i>Amplicon Size</i>	<i>ref</i>
12SV5	TAGAACAGGCTCCTCTAG	TTAGATACCCCACTATGC	57°C	91-105bp	Riaz et al. (2011) (39)
Mam16S	CGGTTGGGGTGACCTCGGA	GCTGTTATCCCTAGGGTAACT	57°C	89-115bp	Taylor (1996) (40)
Fish16S	GACCCTATGGAGCTTAGAC	CGCTGTTATCCCTADRGTAACT	54°C	198-226bp	Deagle et al. (2007) (41), Berry et al. (2017) (42)
12SAH	CTGGGATTAGATACCCCACTAT	CCTTGACCTGTCTTGTAGC	57°C	229-235bp	Cooper (1994) (43)
Kakapo_CR3	CCTAATAGAGTTTTTCAGGGGTGA	CGTATGATTGTCCAGCGAGA	52°C	214 bp	Bergner et al. (2016) (44)

Table S3. Mammal species identified. ^ Upgrade (family -> species). v Downgrade (species -> family).> Inferred (species > species). A: archaeological sites, P: paleontological sites.

<i>Family</i>	<i>Taxon</i>	<i>Common Name</i>	<i>Date introduced</i>	<i>A</i>	<i>P</i>
Muridae	<i>Rattus exulans</i>	Kiore	~AD 1300 (3)	5	6
	<i>Rattus rattus</i>	Black rat	~AD 1870 (3)	-	1
Leporidae	<i>Lepus europaeus</i>	Brown hare	AD 1851 (4)	-	1
	<i>Oryctolagus cuniculus</i>	European rabbit	AD 1838 (4)	-	3
Bovidae	<i>Bos</i> *	Cattle	AD 1814 (4)	10	4
	<i>Capra</i> *	Goat	~AD 1800 (4)	1	-
	<i>Ovis</i> *	Sheep	AD 1773 (4)	4	5
Canidae	<i>Canis lupus familiaris</i> *	Dog/Kuri	~AD 1300 (3)	12	3
Mustelidae	<i>Mustela</i>	Weasel	AD 1885 (3)	-	1
Otariidae	<i>Arctocephalus forsteri</i>	NZ fur seal	<i>native</i>	9	-
	<i>Phocarcos hookeri</i>	NZ sea lion	<i>native</i>	7	-
Phocidae	<i>Hydrurga leptonyx</i>	Leopard seal	<i>native</i>	1	-
	<i>Mirounga leonina</i>	Southern elephant seal	<i>native</i>	8	-
Felidae	<i>Felis</i> *	Cat	~AD 1830 (3)	3	2
Balaenidae	<i>Eubalaena australis</i> ^	Southern right whale	<i>native</i>	1	-
	<i>Balaenoptera physalus</i>	Fin whale	<i>native</i>	1	-
Delphinidae	<i>Orcinus orca</i>	Orca	<i>native</i>	1	-
	<i>Delphininae</i>	True dolphins	<i>native</i>	2	-
Ziphiidae	<i>Ziphius cavirostris</i> ^	Cuvier's beaked whale	<i>native</i>	1	-
Mystacinidae	<i>Mystacina</i> ^	NZ short-tailed bats	<i>native</i>	-	2
Phalangeridae	<i>Trichosurus vulpecula</i>	Common brushtail possum	AD 1858 (4)	-	3

*Common laboratory contaminant

Table S4. Fish and shark species identified. ^ Upgrade (family -> species). v Downgrade (species -> family). > Inferred (species > species). N: North Island sites, S: South Island sites.

Family	Taxon	Common Name	North Island	South Island
Anguillidae	<i>Anguilla australis</i>	Short-finned eel	1	-
Congridae	<i>Gnathophis</i>	Congrid eel	-	1
	<i>Conger</i>	Conger eel	-	2
Carangidae	<i>Trachurus</i>	Jack mackerel	1	-
Cheilodactylidae	<i>Cheilodactylus</i>	-	3	5
Kyphosidae	<i>Scorpis</i>	Sea chub	1	-
Latridae	<i>Latridopsis</i> ^v	-	2	1
	<i>Latris lineata</i>	Striped trumpeter	-	2
Moridae	<i>Pseudophycis</i>	Red cod	-	3
Galaxiidae	<i>Galaxias</i> *	-	-	1
Labridae	<i>Notolabrus</i> ^v	-	1	6
	<i>Pictilabrus</i>	-	1	1
Odacidae	<i>Odax pullus</i>	Green bone	1	3
Mugilidae	<i>Aldrichetta forsteri</i>	Yellow eye mullet	-	3
Ophidiidae	<i>Genypterus blacodes</i>	Ling	-	9
Polyprionidae	<i>Polyprion oxygeneios</i> ^{>}	Hāpuku	3	8
Bovichtidae	<i>Bovichtus</i> ^v		-	1
Nototheniidae	<i>Notothenia angustata</i>	Maori chief	-	1
Sebastidae	<i>Helicolenus</i>	-	-	4
Triglidae	<i>Chelidonichthys kumu</i> [^]	Bluefin gurnard	1	-
Pleuronectidae	<i>Colistium</i> ^v	NZ flounder	1	-
Arripidae	<i>Arripis trutta</i> [^]	Kahawai	2	2
Bramidae	<i>Brama</i>	-	-	1
Centrolophidae	<i>Seriolella brama</i>	Blue warehou	-	1
Gempylidae	<i>Thyrsites atun</i>	Barracouta	2	12
Trichiuridae	<i>Lepidopus caudatus</i>	Silver scabbard fish	-	1
Sparidae	<i>Pagrus auratus</i> [^]	Australasian snapper	3	2
Monacanthidae	Monacanthidae ^v	-	1	1
Hemiramphidae	<i>Hyporhamphus ihi</i> ^{>}	New Zealand piper	1	-
Squalidae	<i>Squalus</i>	Spurdog	-	1

*Found in a palaeontological site

Table S5. Bird species identified. Only lowest taxonomic nodes detected are shown. ^ Upgrade (family -> species). v Downgrade (species -> family). E: endemic, I: introduced, N: native, A: archaeological sites, P: paleontological sites.

Family	Taxon	Common Name	NZ status	Conservation Status	A	P
Sternidae	<i>Chlidonias albobristatus</i>	Black fronted tern	E	Nationally Endangered	1	-
Laridae	<i>Larus</i> sp.	Gulls	-	-	2	-
Scolopacidae	<i>Coenocorypha</i> [^]	Austral snipe	E	Extinct	1	2
Charadriidae	<i>Anarhynchus frontalis</i>	Wrybill	E	Nationally vulnerable	1	1
Columbidae	<i>Columba</i> sp.	Feral pigeon	I	-	-	1
	<i>Hemiphaga novaeseelandiae</i>	NZ pigeon	E	Not threatened	7	2
Accipitridae	<i>Aquila moorei</i>	Haast's Eagle	E	Extinct	-	1
	<i>Circus teauteensis</i>	Eyles harrier	E	Extinct	1	1
Falconidae	<i>Falco novaeseelandiae</i> [^]	NZ falcon	E	Nationally vulnerable	-	1
Anatidae	<i>Cygnus</i> ^{>}	Black swan	-	-	1	1
	<i>Anas</i>	-	-	-	2	4
	<i>Chenonetta</i> ^v	-	-	-	-	7
	<i>Tadorna variegata</i> [^]	Paradise shelduck	E	Not threatened	1	1
Phasianidae	<i>Coturnix</i>	Quail	-	-	1	5
Rallidae	<i>Gallirallus australis</i>	Weka	E	Declining	3	4
	<i>Gallirallus australis greyi</i>	North Island Weka	-	-	-	2
	<i>Aptornis defossor</i>	South Island Adzebill	E	Extinct	-	3
	<i>Porphyrio</i>	Pukeko/Takahe	-	-	-	1
	<i>Fulica</i>	Coot	-	-	-	1
Gruidae	Unknown Gruidae ^v	Unknown crane	-	-	1	-
Meliphagidae	<i>Prothemadera novaeseelandiae</i>	Tui	E	Not threatened	5	3
Acanthisittidae	Acanthisittidae	New Zealand wrens	E	-	-	3
	<i>Xenicus longipes</i>	Bush wren	E	Extinct	-	1
Fringillidae	<i>Carduelis carduelis</i>	European goldfinch	I	Introduced, Naturalized	-	1
Oriolidae	<i>Turnagra capensis</i>	South Island Piopio	E	Extinct	1	4
Callaeatidae	<i>Callaeas cinereus</i>	South Island kokako	E	Extinct	-	5
	<i>Philesturnus carunculatus</i>	South Island saddleback	E	Recovering	2	2
Corvidae	<i>Corvus antipodum</i> [^]	NZ Raven	E	Extinct	-	1
Acanthizidae	<i>Gerygone igata</i>	Grey Warbler	E	Not threatened	1	-
	<i>Mohoua</i>	-	E	Nationally vulnerable	-	1
Locustellidae	<i>Megalurus punctatus</i>	NZ fernbird	E	Declining	-	1
Petroicidae	<i>Petroica macrocephala</i>	Tomtit	E	Not threatened	-	1
	<i>Petroica australis/longipes</i> ^v	NZ robin	E	Declining	-	6
Phalacrocoracidae	<i>Phalacrocorax punctatus</i>	Spotted shag	E	Not threatened	3	-
	<i>Microcarbo melanoleucos</i>	Little pied cormorant	N	Not threatened	1	-
Procellariidae	<i>Pachyptila</i> ^{>}	Prion	N	-	3	-
	<i>Ardenna</i>	Shearwater	N	-	1	-
Diomedeidae	<i>Diomedea</i>	Albatross	N	-	1	-
Psittacidae	<i>Cyanoramphus</i> [^]	NZ parakeet	E	-	5	9
Strigopidae	<i>Nestor meridionalis</i>	Kākā	E	Nationally vulnerable	1	1
	<i>Nestor notabilis</i>	Kea	E	Nationally Endangered	-	3
	<i>Strigops habroptilus</i>	Kākāpō	E	Nationally Critical	-	7
Spheniscidae	<i>Eudyptes pachyrhynchus</i>	Fiordland crested penguin	E	Nationally vulnerable	1	-
	<i>Megadyptes</i> ^v	-	-	-	2	-
	<i>Eudyptula</i>	Little Penguin	N	Declining	1	-
Strigidae	<i>Ninox novaeseelandiae</i>	Morepork	N	Not threatened	-	1
	<i>Ninox albigularis</i>	Laughing owl	E	Extinct	-	1
Apterygidae	<i>Apteryx</i>	Brown kiwi	E	Nationally vulnerable	-	4
	<i>australis/mantelli/rowi</i> ^v	-	-	-	-	-
	<i>Apteryx haastii</i>	Great spotted kiwi	E	Nationally vulnerable	-	2
	<i>Apteryx owenii</i>	Little spotted kiwi	E	Recovering	-	3
Dinornithidae	<i>Dinornis giganteus</i>	South Island giant moa	E	Extinct	1	1
Emeidae	Emeidae sp.	-	E	Extinct	4	6
	<i>Emeus crassus</i> [^]	Eastern moa	E	Extinct	-	1
	<i>Anomalopteryx didiformis</i>	Little bush moa	E	Extinct	2	3
	<i>Megalapteryx didinus</i>	Upland moa	E	Extinct	1	5

Table S6. Reptiles identified. A: archaeological sites, P: paleontological sites.

Family	Taxon	Common Name	NZ status	Conservation status	A	P
Sphenodontidae	<i>Sphenodon punctatus</i>	Tuatara	Endemic	Relict	1	3
Scincidae	<i>Oligosoma</i> ^ sp.	Skinks	Native	-	-	1
Leiopelmatidae	<i>Leiopelma archeyi</i>	Archey's frog	Endemic	Vulnerable	-	1
	<i>Leiopelma hamiltoni</i>	Hamilton's frog	Endemic	Nationally critical	-	1
	<i>Leiopelma markhami</i>	Markham's frog	Endemic	Extinct	-	3

Table S7. Taxa detected in control samples. GB: Grinding blank, EB: Extraction blank, PB: PCR blank.

Control type	Sample prep. batch	Homo sapiens	Sus	Phasianinae
EB1	NZ1	13416	988	-
EB2	NZ1	5991	-	-
PB	NZ1	<i>no amplification</i>		
GB	NZ2	<i>no amplification</i>		
EB	NZ2	<i>no amplification</i>		
GB	NZ3	61137	0	145
PB	NZ3	<i>no amplification</i>		
EB	NZ4	6418	0	0
PB	NZ4	11256	0	0
EB	NZ5	3999	0	0
PB	NZ5	2934	0	0

Table S8. Sample descriptions, blanks.

Sample ID	Sequencing batch name	12Sv5		Mam16S		Fish16S		12Sah	
		Reads	OTUs	Reads	OTUs	Reads	OTUs	Reads	OTUs
EB1	NZ1	2266	2	12138	4	-	-	-	-
EB2	NZ1	-	-	5991	1	-	-	-	-
PB	NZ1	-	-	-	-	-	-	-	-
GB	NZ2	-	-	-	-	-	-	-	-
EB	NZ2	-	-	-	-	-	-	-	-
GB	NZ3	61137	1	-	-	-	-	145	1
PB	NZ3	-	-	-	-	-	-	-	-
EB	NZ4	-	-	6418	1	-	-	-	-
PB	NZ4	-	-	11256	1	-	-	-	-
EB	NZ5	3999	2	-	-	-	-	-	-
PB	NZ5	2934	1	-	-	-	-	-	-

Table S9. Bulk bone pool descriptions.

Unique bone grinds	Sample preparation batch	Bulk bone pool	n bones	Amplifications			
Ardenest	NZ1	-	106	Ard_NZ1			
Awamoa	NZ1	PoolA	159	AM_A_NZ5	AM_B_NZ5		
Awamoa	NZ3	PoolB	50	AM_NZ3			
Cobden Cave	NZ1	PoolA	98	Cob_NZ1	Cob_II_NZ3	Cob_B_NZ4	Cob_C_NZ4
Cobden Cave	NZ4	PoolB	9	Cob_A_NZ4			
Cooks Cove	NZ3	PoolA	50	CC_A_NZ3			
Cooks Cove	NZ3	PoolB	50	CC_B_NZ3			
Cooks Cove	NZ3	PoolC	50	CC_C_NZ3			
Cooks Cove	NZ2	PoolD	27	CC_NZ2			
D'Urville Island	NZ4	-	34	Dur_NZ4			
Dashing Rocks - Timaru	NZ1	-	100	Tim_NZ1			
Eagles Roost	NZ4	PoolA	2	EaR_A_NZ4			
Eagles Roost	NZ1	PoolB	100	EaR_B_NZ4			
Falcon Site	NZ1	-	92	Fal_NZ1			
Finsch's Folly	-	PoolA	50	FF_MB2125	FF_MB2126	FF_MB2127	
Finsch's Folly	-	PoolB	50	FF_MB2128	FF_MB2129	FF_MB2130	
Finsch's Folly	-	PoolC	50	FF_MB2131	FF_MB2132	FF_MB2133	
Finsch's Folly	-	PoolD	50	FF_MB2149	FF_MB2150	FF_MB2151	
Finsch's Folly	-	PoolE	50	FF_MB2152	FF_MB2153	FF_MB2154	
Fyffe Site	NZ1	-	102	Fyf_A_NZ5	Fyf_B_NZ5		
Gordons Valley Station	NZ1	-	91	Gor_NZ1			
Goulands Downs Cave	NZ1	-	100	Gou_NZ1			
Graveyard	NZ1	PoolA	107	GY_A_NZ1			
Graveyard	NZ1	PoolB	108	GY_B_A_NZ5	GY_B_B_NZ5		
Kahukura	NZ3	PoolA	50	KK_A_NZ3			
Kahukura	NZ3	PoolB	50	KK_B_NZ3			
Kahukura	NZ3	PoolC	50	KK_C_NZ3			
Kahukura	NZ3	PoolD	50	KK.fb_NZ3			
Kahukura	NZ3	PoolE	50	KK.fv_NZ3			
Kawatiri	NZ1	-	114	Kaw_NZ1			
Kids Cave	NZ1	-	103	Kid_NZ1			
Long Beach	NZ1	PoolA	113	Lon_NZ1			
Long Beach	NZ3	PoolB	50	Lon_NZ3			
Main Road 27	NZ1	-	43	R27_A_NZ5	R27_B_NZ5		
Martinborough Cave #1	NZ2	PoolA	50	MarC_NZ2			
Martinborough Cave #1	NZ4	PoolB	39	MarC_NZ4			
Moabone Point Cave	NZ1	-	86	Rmo_A_NZ5	Rmo_B_NZ5		
Moncks Cave	NZ1	-	164	MonC_NZ1			
Mt Cookson	NZ1	-	106	MtC_NZ1			
Omaio	NZ1	-	114	Oma_A_NZ5	Oma_B_NZ5		
Poukawa XII	NZ1	PoolA	113	Pou_NZ1	Pou_C_NZ2		
Poukawa XII	NZ2	PoolB	50	Pou_B_NZ2			
Predator Cave	NZ1	-	103	Pre_NZ1			
Shag Point	NZ1	PoolA	122	SP_C_NZ2			
Shag Point	NZ2	PoolB	50	SP_B_NZ2			
St Clair	NZ3	PoolA	50	SC_A_NZ3			
St Clair	NZ3	PoolB	50	SC_B_NZ3			
St Clair	NZ3	PoolC	50	SC_C_NZ3			
Takaka Fossil cave	NZ1	-	90	Tak_NZ1			
Te Waka #1	NZ1	-	103	TeW_A_NZ5	TeW_B_NZ5		
Tokenui	NZ3	PoolA	50	TN_A_NZ3			
Tokenui	NZ3	PoolB	50	TN_B_NZ3			
Tokenui	NZ3	PoolC	50	TN_C_NZ3			
Tokenui	NZ1	PoolD	105	TN_NZ1			
Tokerau Beach	NZ1	PoolA	105	Tok_A_NZ5	Tok_B_NZ5		
Tokerau Beach	NZ1	PoolB	100	Tok_C_NZ2			
Tokerau Beach	NZ2	PoolC	37	Tok_B_NZ2			
Tumbledown Bay	NZ1	-	126	Tum_NZ1			
Waikanae	NZ1	-	102	Kap_A_NZ5	Kap_B_NZ5		
Waimataitai	NZ1	-	98	Wm_NZ1			
Wairau Bar	NZ1	PoolA	103	Wai_A_NZ5	Wai_B_NZ5		
Wairau Bar	NZ1	PoolB	85	Wai_B_NZ1			
Wairau Bar	NZ3	PoolC	50	Wai_A_NZ3			
Wairau Bar	NZ3	PoolD	50	Wai_B_NZ3			
Wairau Bar	NZ3	PoolE	50	Wai_C_NZ3			
Wakanui	NZ1	-	123	Wak_NZ1			
Watsons Beach	NZ3	PoolA	50	Wat_A_NZ3			
Watsons Beach	NZ3	PoolB	50	Wat_B_NZ3			
Watsons Beach	NZ3	PoolC	50	Wat_C_NZ3			
Whalers Bay Cave	NZ1	-	144	Wha_NZ1			

Table S10. Post filtering DNA amplification descriptions

Sample ID	Sequencing batch name	12Sv5		Mam16S		Fish16S		12Sah	
		Reads	OTUs	Reads	OTUs	Reads	OTUs	Reads	OTUs
AM_A_NZ5	NZ5	3008	6	5059	2	-	-	905	1
AM_B_NZ5	NZ5	2699	5	2938	3	-	-	447	3
AM_NZ3	NZ3	4527	2	34081	6	-	-	-	-
Ard_NZ1	NZ1	6414	17	10341	6	-	-	-	-
CC_A_NZ3	NZ3	451	8	-	-	549	2	-	-
CC_B_NZ3	NZ3	968	5	21	2	262	2	-	-
CC_C_NZ3	NZ3	1315	17	168	3	1495	10	-	-
CC_NZ2	NZ2	660	6	552	2	-	-	-	-
Cob_A_NZ4	NZ4	3955	5	758	3	-	-	2096	4
Cob_B_NZ4	NZ4	2303	11	541	4	-	-	3992	5
Cob_C_NZ4	NZ4	3252	8	3054	4	-	-	4158	7
Cob_H_NZ3	NZ3	-	-	8853	5	-	-	14817	3
Cob_NZ1	NZ1	3069	10	681	6	-	-	306	6
Dur_NZ4	NZ4	10229	10	5695	3	-	-	-	-
EaR_A_NZ4	NZ4	8539	1	-	-	-	-	1391	3
EaR_B_NZ4	NZ4	7204	8	2372	3	-	-	3112	2
Fal_NZ1	NZ1	5694	10	6617	2	-	-	6	1
FF_MB2125	Grealy et al. 2015	-	-	-	-	-	-	2248	6
FF_MB2126	Grealy et al. 2015	-	-	-	-	-	-	3988	6
FF_MB2127	Grealy et al. 2015	-	-	-	-	-	-	969	5
FF_MB2128	Grealy et al. 2015	-	-	-	-	-	-	3368	7
FF_MB2129	Grealy et al. 2015	-	-	-	-	-	-	6515	5
FF_MB2130	Grealy et al. 2015	-	-	-	-	-	-	10751	7
FF_MB2131	Grealy et al. 2015	-	-	-	-	-	-	2210	5
FF_MB2132	Grealy et al. 2015	-	-	-	-	-	-	4332	11
FF_MB2133	Grealy et al. 2015	-	-	-	-	-	-	4747	6
FF_MB2149	Grealy et al. 2015	-	-	-	-	-	-	2130	9
FF_MB2150	Grealy et al. 2015	-	-	-	-	-	-	5550	5
FF_MB2151	Grealy et al. 2015	-	-	-	-	-	-	3773	7
FF_MB2152	Grealy et al. 2015	-	-	-	-	-	-	10548	6
FF_MB2153	Grealy et al. 2015	-	-	-	-	-	-	9596	7
FF_MB2154	Grealy et al. 2015	-	-	-	-	-	-	7866	5
Fyf_A_NZ5	NZ5	3689	15	2436	4	2305	6	2346	2
Fyf_B_NZ5	NZ5	3051	18	3588	9	2515	10	2774	1
Gor_NZ1	NZ1	5772	16	11592	2	-	-	-	-
Gou_NZ1	NZ1	6584	17	459	7	-	-	-	-
GY_A_NZ1	NZ1	5215	8	136	1	-	-	22	1
GY_B_A_NZ5	NZ5	1893	10	782	4	-	-	1628	4
GY_B_B_NZ5	NZ5	2081	6	637	4	-	-	1750	7
Kap_A_NZ5	NZ5	2755	6	-	-	3343	7	-	-
Kap_B_NZ5	NZ5	5134	7	-	-	2777	6	-	-
Kaw_NZ1	NZ1	44	1	12	1	-	-	-	-
Kid_NZ1	NZ1	2697	6	4522	5	-	-	-	-
KK_A_NZ3	NZ3	6546	10	16849	15	116	4	-	-
KK_B_NZ3	NZ3	4554	10	21993	18	918	3	-	-
KK_C_NZ3	NZ3	5536	8	23594	18	1100	4	59305	3
KK.fb_NZ3	NZ3	8722	4	7994	2	322	4	-	-
KK.fv_NZ3	NZ3	7858	7	546	1	389	5	-	-
Lon_NZ1	NZ1	7746	7	10461	9	12973	4	-	-
Lon_NZ3	NZ3	1514	2	10176	2	-	-	-	-
MarC_NZ2	NZ2	26831	15	48400	11	-	-	11672	8
MarC_NZ4	NZ4	11096	11	7490	3	-	-	750	4
MonC_NZ1	NZ1	5635	18	2796	9	5096	15	22	2
MtC_NZ1	NZ1	4538	11	11699	3	-	-	5	1
Oma_A_NZ5	NZ5	1736	3	-	-	2804	7	-	-
Oma_B_NZ5	NZ5	1895	6	1259	1	2999	8	-	-
Pou_B_NZ2	NZ2	-	-	5	1	-	-	-	-
Pou_B_NZ2_NZ2	NZ2	15172	1	-	-	-	-	-	-
Pre_NZ1	NZ1	6173	15	12078	3	-	-	5	1
R27_A_NZ5	NZ5	4927	5	2930	2	-	-	559	5
R27_B_NZ5	NZ5	3267	4	2964	2	-	-	524	9
Rmo_A_NZ5	NZ5	3454	9	4764	6	3125	3	3533	1
Rmo_B_NZ5	NZ5	2909	10	3520	8	2444	3	2997	1
SC_A_NZ3	NZ3	7132	9	33819	6	-	-	40120	7
SC_B_NZ3	NZ3	9198	11	40792	8	-	-	21233	6
SC_C_NZ3	NZ3	6742	9	36300	5	19	1	70022	3
SP_B_NZ2	NZ2	32154	12	25572	2	22645	4	-	-
SP_C_NZ2	NZ2	32045	13	25187	3	18159	9	-	-
Tak_NZ1	NZ1	2815	19	1615	6	-	-	25	2
TeW_A_NZ5	NZ5	3042	14	596	3	-	-	2976	8
TeW_B_NZ5	NZ5	2378	15	546	7	-	-	2695	12
Tim_NZ1	NZ1	2010	5	295	2	5618	2	-	-
TN_A_NZ3	NZ3	8090	6	40288	3	450	8	-	-
TN_B_NZ3	NZ3	5549	10	41907	5	925	5	-	-
TN_C_NZ3	NZ3	4508	8	35435	3	1121	5	54818	1
TN_NZ1	NZ1	6078	9	10497	1	5807	5	-	-
Tok_A_NZ5	NZ5	2967	2	-	-	-	-	-	-
Tok_B_NZ2	NZ2	-	-	-	-	-	-	12948	1
Tok_B_NZ2_NZ2	NZ2	11633	1	-	-	-	-	-	-
Tok_B_NZ5	NZ5	3126	3	15	2	-	-	-	-
Tok_C_NZ2	NZ2	-	-	714	1	-	-	2176	1
Tok_C_NZ2_NZ2	NZ2	30674	3	-	-	-	-	-	-
Tum_NZ1	NZ1	7014	10	1149	4	6175	3	60	1

Wai_A_NZ3	NZ3	571	4	88	3	1501	2	32096	5
Wai_A_NZ5	NZ5	3144	13	1381	7	-	-	2896	2
Wai_B_NZ1	NZ1	6919	7	5916	5	6593	3	-	-
Wai_B_NZ3	NZ3	1575	6	14026	3	1164	1	-	-
Wai_B_NZ5	NZ5	4733	17	1212	5	1978	4	1856	4
Wai_C_NZ3	NZ3	3513	2	18733	3	-	-	-	-
Wak_NZ1	NZ1	1378	4	29	1	-	-	-	-
Wat_A_NZ3	NZ3	6056	13	36765	10	1389	5	24892	7
Wat_B_NZ3	NZ3	6895	8	9268	7	527	3	21000	5
Wat_C_NZ3	NZ3	7061	9	22123	4	1211	1	18961	4
Wha_NZ1	NZ1	3954	14	6674	7	7775	11	6	1
Wm_NZ1	NZ1	7232	7	182	1	6616	3	-	-

Additional data table S11 (separate file)

Raw taxonomic assignments for all sites studied along with information on reassigned taxa. Numbers in each field of the table designate the number of separate subsamples in which a given taxa was identified in each site.

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