

Ancient DNA of New Zealand's Extinct Avifauna

Using next-generation sequencing
(NGS) to research the paleoecology of
the megaherbivore moa (Aves:
Dinornithiformes †) and the
phylogenetics of the enigmatic gruiform
Aptornis (Aves: Aptornithidae †)

A thesis submitted in fulfilment for the requirements
for the degree of Master of Philosophy

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“... a symphony of ‘the most tunable silver sound imaginable’. Aotearoa’s multitudes of birds performed that symphony each dawn for over 60 million years. It was a glorious riot of sound with its own special meaning, for it was a confirmation of the health of a wondrous and unique ecosystem. To my great regret, I arrived in New Zealand in the late twentieth century only to find most of the orchestra seats empty. Walking through the ancient forest, whose still-living trees were once browsed by moa, I heard nothing but the whisper of leaves blowing in the wind. It was like the rustle of the last curtain fall on an orchestra that will be no more” Tim Flannery (*The Future Eaters*)



Image: skulls of *Aptornis defossor* (South Island adzebill, top) and *Pachyornis elephantopus* (heavy-footed moa, bottom) from Richard Owen, *Memoirs on the extinct wingless birds of New Zealand, with an appendix on those of England, Australia, Newfoundland, Mauritius, and Rodriguez*, 1879

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Abstract

As a direct result of human activity and other environmental changes, considerable ecological changes and species extinctions have occurred on most terrestrial environments during the last 50,000 years. Therefore, correctly interpreting naturally occurring biogeography or ecology is limited without including historical information. Detailed insights into past environments or extinct species can be provided by ancient DNA (aDNA), yet this field has long been limited by the degraded nature of ancient genetic material. Recent developments and the increasing availability of next-generation sequencing (NGS), is now allowing for a breakthrough in aDNA studies. It is now possible to obtain entire genomes from long-extinct organisms, and sequence high-depth 'environmental DNA' representative of prehistoric environments.

New Zealand (NZ) was the last of the large landmasses (except Antarctica) to be colonised by humans, approximately 700 years ago. Furthermore, the long isolation of NZ from Gondwana (from which it separated between ~85-52 Ma) resulted in an ancient, ecologically complex and biogeographically unique biota. Due to a near-absence of mammals this biota was dominated by a diverse array of endemic bird species, of which nearly 50% are now extinct due to human activity. Nonetheless, the recent age of NZ's extinctions, combined with a temperate climate, has permitted an unparalleled degree of preservation of the pre-human ecosystem. By use of aDNA and NGS methods, NZ's biota may now be understood in relative completion.

This thesis uses aDNA NGS methods to research a select number extinct bird species. Chapters Two and Three investigate environmental DNA of avian coprolites (paleofaeces), most of which originate from four species of the megaherbivore ratite moa (*Dinornithiformes*). It is identified for the first time that the extinct moa consumed and dispersed species of mycorrhizal fungi important in natural forest dynamics. Phylogenetics of sequenced moa parasite DNA found rare evidence for a higher taxonomic level co-extinction event: between moa, and an identified genus or family of moa-specific nematodes. Finally, differences in parasite, plant and fungal DNA assemblages greatly support divergent behaviour strategies between each moa species. I also analyse shotgun DNA and DNA-RNA hybridization enrichment data on avian coprolites representative of rich deposits in semi-arid areas of the central South Island. I find that these samples have been greatly affected by thermal-based DNA damage and/or formation of organic compounds inhibiting DNA amplification. Future approaches aiming to resolve these issues are discussed.

Chapter Four investigates the phylogeny one of NZ's most poorly understood large bird taxa, the adzebills (*Aptornis*, *Aptornithidae*). Sequencing of near-complete complete mitochondrial genomes of both recently extinct species, resolves placement of these birds within the superfamily Ralloidea (*Gruiformes*). This in turn elucidates evolutionary details into the global radiation of Ralloid birds and the *Aptornis* lineage in NZ.

The final Fifth chapter summarizes the results of these diverse studies and how they interrelate, and concludes the thesis by discussing future related aDNA projects to be undertaken on NZ's ancient ecosystem.

Contents

Chapter One: General Introduction	11
1.1 Foreword and Background	12
1.2 NZ's lost avifauna	14
1.3 NZ's natural history	19
1.4 Aims, objectives and outline of this thesis	23
Chapter Two: Metabarcoding ancient metagenomes of animal coprolites: revealing the lost ecological interactions of NZ's extinct ratite moa and critically endangered kakapo parrot	35
Statement of authorship	36
2.1 Significance	38
2.2 Abstract	38
2.3 Introduction	39
2.4 Results	41
2.4.1 <i>Site and sample information</i>	41
2.4.2 <i>Total Diversity</i>	42
2.4.3 <i>Fungal aDNA and evidence of dietary fungi</i>	42
2.4.4 <i>Plant aDNA, including fern and moss taxa</i>	43
2.4.5 <i>Parasite aDNA and moa-parasite co-extinction</i>	45
2.5 Discussion	46
2.5.1 <i>Improvements of NGS metabarcoding over Sanger sequencing</i>	47
2.5.2 <i>New dietary taxa, niche partitioning and dispersive roles of moa</i>	47
2.5.3 <i>Indirect evidence for moa interactions and migratory behaviour</i>	48
2.5.4 <i>Extinction of host-specific parasites</i>	49
2.5.5 <i>Conclusions</i>	50
2.6 Methods (note)	50
2.7 Figures	51
2.8 References	54

Chapter Two: Supplementary information	58
S2.1 Methods	58
<i>S.2.1.1 Site and specimen details</i>	58
<i>S.2.1.2 DNA extraction and amplification</i>	59
<i>S.2.1.3 QIIME analyses</i>	59
<i>S.2.1.4 BLASTn and phylogenetic identifications</i>	61
S2.2 Figures	63
S2.3 References	82
Chapter Three: Shotgun metagenomes of six avian coprolites from NZ	85
Statement of authorship	86
3.1 Abstract	88
3.2 Introduction	88
3.3 Methods	91
<i>3.3.1 Materials</i>	91
<i>3.3.2 Extraction and amplification</i>	92
<i>3.3.3 Analysis and identification</i>	93
<i>3.3.4 Host DNA identification</i>	93
3.4 Results	94
<i>3.4.1 Sequencing depth and read proportions</i>	94
<i>3.4.2 Host DNA content</i>	95
<i>3.4.3 Eukaryotic taxon assignments</i>	95
3.5 Discussion	97
<i>3.5.1 Endogenous DNA content</i>	97
<i>3.5.2 Ecological inferences</i>	97
<i>3.5.3 Comparison between Metabarcoding / Shotgun datasets</i>	98
<i>3.5.4 DNA preservation in coprolites and future directions</i>	99
3.6 Figures	101
3.7 References	107

Chapter Four: Two near-complete mitochondrial genomes of NZ’s extinct, enigmatic adzebills (Aves: Aptornithidae †) resolve taxonomic placement within the superfamily Ralloidea (Gruiformes), and identifies biogeographic link between NZ and the Afrotropics	111
Statement of authorship	112
4.1 Abstract	113
4.2 Introduction	113
4.3 Methods	117
4.3.1 <i>Specimen Sampling and DNA extraction</i>	117
4.3.2 <i>DNA sequencing and amplification</i>	118
4.3.3 <i>Genome assembly</i>	119
4.3.4 <i>Phylogenetic analyses and taxon sampling</i>	120
4.3.5 <i>Fossil calibrations and molecular clock analyses</i>	122
4.4 Results	124
4.4.1 <i>Tree topology and biogeographic assessment</i>	124
4.4.2 <i>Divergence times</i>	125
4.5 Discussion	126
4.5.1 <i>Age, origins and Phylogeny of Aptornis</i>	126
4.5.2 <i>Ralloidea topology and taxonomy</i>	127
4.5.3 <i>Divergence between North and South Island Aptornis</i>	129
4.5.4 <i>Macroevolution and ecological niche of Aptornis</i>	129
4.6 Figures	132
4.7 References	142
Chapter Five: General Discussion	149
5.1 General Summary, significance, and limitations of this thesis	150
5.2 Ancient DNA of late Quaternary animal coprolites from New Zealand	152
5.2.1 <i>Metabarcoding and metagenomic studies</i>	152
5.2.2 <i>Prehistoric avian dispersal of New Zealand’s mycorrhizal fungi</i>	153
5.2.3 <i>Parasite-host co-extinction</i>	155
5.2.4 <i>Paleoecological and parasitic changes of the kakapo parrot</i>	156
5.2.5 <i>Further aDNA coprolite studies</i>	157

5.3	The adzebills: an evolutionary and ecological enigma	157
	5.3.1 <i>Phylogenetic analyses and additional taxon sampling</i>	157
	5.3.2 <i>Population genetics and paleoecology</i>	158
5.4	Evolution and paleoecology in New Zealand: outstanding questions	159
	5.4.1 <i>Biogeography of New Zealand in the Late Paleogene-Neogene</i>	159
	5.4.2 <i>Discordance with fossils and present phylogenetics: evolution of New Zealand's rail and waterfowl faunas</i>	160
	5.4.3 <i>New Zealand's seabird communities</i>	160
	5.4.4 <i>New Zealand's lost invertebrate and herpetological faunas</i>	161
5.5	Concluding Remarks	161
5.6	References	163