

# **Molecular approaches define the interactions and impacts of Tasmania's native, introduced and invasive marsupials.**

**Catriona D. Campbell**

B.Env.Sc. (Hons), Deakin University, Melbourne, Australia

Institute for Applied Ecology, University of Canberra  
ACT, Australia  
November, 2017



A thesis submitted in fulfilment of the requirements for the degree of  
Doctor of Philosophy in Applied Science at the University of  
Canberra, Australia

## Abstract

Diversification of new species is driven by genome evolution, which is influenced by demographic processes such as gene flow, genetic drift and geographic isolation. These processes do not act alone but work together. Modern molecular technologies mean that we now have unparalleled power to monitor the role that humans may play in species introductions and understand the ecological processes affecting both introduced and native species. Here I have used a range of molecular and statistical techniques to study the ecological impacts of three separate invasion events involving Tasmania and how these invasions impact the genomes of invaders and the diet and interactions of the native conspecifics.

Firstly, I used historical records, genetic data and Bayesian analysis to uncover the provenance of a once thought native marsupial in Tasmania, Australia. This species, the sugar glider (*Petaurus breviceps*), has recently been found to prey upon an endangered parrot and knowledge of the alien provenance of this marsupial predator will allow managers to correct legislation dealing with its current protection in the state. Secondly, I used genotype-by-sequencing to reveal population structure of an introduced marsupial in Hawkes Bay, New Zealand. Multiple introductions of the common brushtail possum (*Trichosurus vulpecula*) from mainland Australia & Tasmania into the area have generated a novel genetic form and here I show how the current population structure aligns closely with the introduction histories of the area. This reveals that the introduced Hawkes Bay population is not a single interbreeding population but at least four sub-populations in a relatively small geographic area. Thirdly, I identified two informative molecular barcode markers for the identification of Diprotodontia in Tasmania from unknown DNA sequences. My research highlights the need for a localised DNA reference database to be developed for successful identification of unknown sequences in metabarcoding studies. I showed that uncharacterised intraspecific genetic variation can increase the failure rate of species ID. Thus, I identified the *16sMam* and *12sV5* markers which give the highest assignment to species level for mainland Australian and Tasmanian Diprotodontia species in DNA metabarcoding studies, when used in combination. Lastly, I used high throughput sequencing of predator scats to look at predator and prey interactions across time and space, in the north of Tasmania. This ecosystem is changing with an incursion of disease affecting the apex predator, Tasmanian devil (*Sarcophilus harrisi*), and so it provides a unique opportunity to study ecosystem changes using non-invasive sampling techniques. My research showed the proportion of scats

detected for each predator significantly changed across space, with devil scat detections increasing in an area where the population is thought to be decreasing. There is a high amount of dietary overlap between the introduced predator, feral cat (*Felis catus*), and the smaller *Dasyurus spp.* found in Tasmania. Any interaction between devils and cats is not a competitive one, with a significant difference being found between the two species diets. There was no detectable difference in prey species diversity for cats or quolls when devils were found within the same survey unit.

My research highlights the need for a thorough understanding of both invasive and native species molecular evolution and the ecological processes which have affected the individual populations and may have influenced their genetics. Local genetic knowledge will allow more effective and efficient management protocols to be developed and deployed because we are able to understand the biological mechanics of introduced species at a deeper level.

## ACKNOWLEDGEMENTS

I'd like to start by thanking my supervisory team, Team Poo leaders: Professor Stephen Sarre (IAE), Dr Bernd Gruber (IAE), Dr Anna MacDonald (IAE/ANU) and Dr Stephen Harris (Invasive Species Branch, DPIPWE); and Dr Clare Holleley (IAE/CSIRO) who came on board a little later after Steve Harris retired from DPIPWE. Well, what can I say? I have learned so much from each of you I can't even begin to list everything. Each one of you brought something different to my team; strengths, knowledge, personalities and without each person I would not have been able to finish this huge project! Thank you so much.

I would like to thank my major funding body, the Invasive Animals Cooperative Research Centre who funded my project and me personally as well as supplying training and industry connections which have put me ahead of other PhD candidates in the race for a job. But I would chiefly like to single out Tony Buckmaster who was given the job of looking after us students and unfortunately for him that meant he was stuck with me. Tony, honestly, you have been the most wonderful support over the years. You are so good at your job and the understanding you've passed out to me on numerous occasions is second to none. I will never forget your offer of help to look after Greta when she was bitten by a snake and after losing Boris in the same incident just two weeks before I left on 4 months of interstate field work, not to mention all the other times you looked out for me. Thank you so very much.

To the other Team Poo'ers Elodie Modave, Candida Barclay and Elise Dewar, thank you. Dida, you're little emails of excitement and encouragement in the last few weeks leading up to me finishing were invaluable! But particularly Elise, we have forged the most special of friendships. We should have met many times over the last ten years but I think holding out until December 2013 was a stroke of genius by the universe. Thank you for being a never ending light in my life. I literally could not have done this without you, both personally and professionally, you're always there for me.

Thank you to the Holsworth Research Endowment who funded my final chapter. This was the biggest chapter in both size and monetary costs and it would not have been possible without the financial backing of the Endowment.

To all the people I was lucky enough to collaborate with, Dejan Stojanovic, Kathryn Medlock, Phil Cowan, thank you for the learning experience and I hope we can continue to work together in the future. Each chapter has its own acknowledgements at the end as there were many tissue collections, lab techs, museums staff, researchers and statistical geniuses who helped along the way.

The Institute for Applied Ecology is a wonderful place to work with so much support from all facets of the department. I would particularly like to thank Barbara Harris who helped me endlessly with admin support, finances and anything else I might need. Your help and support was greatly appreciated. Val Caron and Elise Furlan, both of you let me sit in your offices and have a cry whenever I needed it as well as offering mountains of advice and hugs on tap of which I could not have done without! Everyone in the WGL labs, all great people. Thanks to Matt Young, Sam Venables and Sumaiya Quasim for all the help in the lab, particularly at the start when I had very little idea what I was doing. Thanks to Peter Unmack for all the advice on phylogenetic studies, Arthur Georges for regular advice on just about everything, Di Gleeson for just being you, Janine Deakin for general support and always a happy face and Lla for the great chats in your office.

Thanks go out to Luc Small, formally of Intersect. Luc helped me tirelessly with our remote scat server and loading programs. Thanks Luc!

To all the PhD students I met along the way, thanks for the support, but particularly Margi Sweeny, Sally Hatton and Alan Couch, mates from the start and hopefully mates on into our futures.

To my friends on the outside, thank you for not leaving me! To my besties Jac and Carly, thanks for always being at the other end of the phone when I needed a chat and not getting mad at me when I couldn't make it back to Melbourne for visits all that often, love you both. The Canberra crew, Sarah, Laura, Kristi and Kenk, our random dinners and parties were life savers for me! To the home crew, Annie, Steve, Darryl and particularly Heidi Reid, thanks for coming over and cooking me dinner, taking me out for food and social skills, playing with Greta when I couldn't and making me laugh.

Andrew, you came along late in the piece but you played a most integral part of this process for me. You gave me both personal support and professional support and for that I am eternally grateful. Your calm disposition and genius R knowledge kept me going on days when I didn't think I could continue.

To my wonderful furry babies; Herbert, Greta and Boris, you absolutely tested my patience at times but I wouldn't have it any other way. Without knowing it you supplied me with love and support, and an excuse to get away from my desk or escape the lab. Boris, you broke my heart when you left but I will always remember you and your goofy personality. Herbert, thank you for hanging in there until I finished, I absolutely could not have got through this without you! And Greta, what can I say, you are the most wonderful dog a girl could ask for. Thank you.

Lastly I would like to thank my parents, Heather and Rob Campbell, without whom I could never have completed this project. You allowed me, my two (large) dogs and aging cat to stay with you for what was meant to be a couple of days a week for three years but turned into, at times, every day for weeks and months on end while I worked in the trace lab. You made the considerable effort of living away from my home much easier, not to mention your endless support now and throughout my life. Thank you, love you both.

## Table of Contents

Abstract.....	iii
FORM B.....	v
ACKNOWLEDGEMENTS.....	vii
LIST OF TABLES.....	xv
LIST OF FIGURES.....	xvii
Chapter 1.....	1
General Introduction.....	1
Genetic approaches for identifying and detecting fauna.....	2
Biogeographical history of Greater Australia.....	3
Australia's marsupial diversity.....	5
Tasmanian predators and their diets.....	6
Project description.....	8
Chapter 2.....	11
When is a native species invasive? Incursion of a novel predatory marsupial detected using molecular and historical data.....	11
KEYWORDS: Introduced species, invasive species, mtDNA, native species, range expansion, sugar glider.....	11
ABSTRACT.....	14
INTRODUCTION.....	15
METHODS.....	19
Developing and testing introduction scenarios.....	19
Historical records.....	20
Tissue sample collection.....	20
DNA extraction and PCR amplification.....	20
Sequencing.....	21
Phylogenetic analysis.....	22
Bayesian analysis of population history.....	22
RESULTS.....	23
Historical records.....	23
Phylogenetic analysis.....	25
Bayesian analysis of population history.....	28
DISCUSSION.....	28
ACKNOWLEDGEMENTS.....	31

DATA ACCESSIBILITY STATEMENT .....	31
Chapter 3.....	33
The genetic consequences of repeated introductions during invasion: the hybridisation of two subspecies of brushtail possum in New Zealand .....	33
ABSTRACT .....	36
INTRODUCTION.....	37
METHODS.....	40
Sample selection .....	40
Genotype-by-sequencing .....	42
Analysis of genotype-by-sequencing data .....	42
RESULTS.....	43
SNP Genotyping .....	43
DISCUSSION .....	53
ACKNOWLEDGEMENTS .....	57
DATA ACCESSIBILITY STATEMENT .....	57
ETHICS STATEMENT .....	57
AUTHORS CONTRIBUTION STATEMENT .....	57
FUNDING STATEMENT .....	57
Chapter 4.....	59
<i>In silico</i> evaluation of genetic markers for DNA metabarcoding: is cytochrome c oxidase 1 the best option for Australian marsupials?.....	59
KEYWORDS: <i>DNA metabarcoding, Mitochondrial DNA, COI, 12s, 16s, Marsupials, Diprotodontia, environmental DNA</i> .....	59
ABSTRACT .....	62
INTRODUCTION.....	63
METHODS.....	66
Selection of candidate metabarcoding primers.....	66
Construction of candidate reference databases.....	67
Evaluation of primer utility for amplification of mammal DNA .....	69
Evaluation of the diagnostic ability of each primer set .....	70
Phylogenetic evaluation of candidate metabarcoding markers .....	70
RESULTS.....	71
Construction of custom reference DNA databases.....	71
Evaluation of primer utility for amplification of mammal DNA .....	71

Evaluation of the diagnostic ability of each primer set .....	79
Phylogenetic evaluation of candidate metabarcoding markers .....	81
DISCUSSION .....	84
ACKNOWLEDGEMENTS .....	85
DATA ACCESSIBILITY STATEMENT .....	86
ETHICS STATEMENT .....	86
AUTHORS CONTRIBUTION STATEMENT .....	86
FUNDING STATEMENT .....	86
Chapter 5 .....	87
The devil’s in the diet: a DNA metabarcoding study of dietary overlap in native and introduced predators.....	87
KEYWORDS <i>Metabarcoding, high throughout sequencing, species identification, ecosystem changes, mitochondrial DNA</i> .....	87
ABSTRACT .....	90
INTRODUCTION.....	91
METHODS.....	95
Scat survey.....	95
Custom reference DNA database .....	97
Predator identification .....	97
Scat DNA extraction and quantitation for metabarcoding .....	99
Amplicon PCR amplification and metabarcoding.....	100
Analysis of scat DNA data .....	101
RESULTS.....	102
Scat survey.....	102
Custom reference DNA database .....	102
Predator identification .....	102
Analysis of scat DNA data .....	105
DISCUSSION .....	112
DATA ACCESSIBILITY STATEMENT .....	118
ETHICS STATEMENT .....	118
AUTHORS CONTRIBUTION STATEMENT .....	119
FUNDING STATEMENT .....	119
Chapter 6 .....	121
Synopsis .....	121

Characterisation of genetic diversity within two introduced populations of marsupials and the implications for management of native and invasive species .....	121
Evaluation of candidate markers to identify Tasmanian marsupials from environmental DNA (eDNA) .....	122
Characterisation of mammalian predator diets and prey assemblage in Tasmania.....	123
Implications of this work for management of native and invasive species and future research.....	123
Implications of this work for eDNA analysis .....	124
References.....	127
Supplementary material .....	153
CHAPTER 2.....	153
CHAPTER 3.....	183
CHAPTER 4.....	191
CHAPTER 5.....	203

## LIST OF TABLES

<b>Table 3.1:</b> Pairwise F <sub>st</sub> estimates among four groups of possums in the Hawkes Bay region of New Zealand (A = mainland ancestry, B = mixed ancestry, C = Tasmanian ancestry (1) and D = Tasmanian ancestry (2)).	47
<b>Table 3.2:</b> The proportion of fixed differences, alleles which are fixed within a population, observed in comparisons between brushtail possums from Hawkes Bay, New Zealand, and from the two subspecies of Australian possum that are thought to have founded the Hawkes Bay population.	49
<b>Table 4.1:</b> Primer sets used to test specificity for a Marsupialia metabarcoding study.	67
<b>Table 5.1:</b> The numbers of scat samples analysed during each stage of this study. Scats were collected from two regions of Tasmania during three different surveys.	104
<b>Table S2.1:</b> Metadata for published mtDNA sequences and tissue samples collected within this study (*) and including published sequences.	157
<b>Table S2.2:</b> <i>Petaurus breviceps</i> occurrences with locality data recorded in Tasmania, 1845 – 2015.	165
<b>Table S2.3:</b> Major fauna collecting and survey expeditions to Tasmania.	173
<b>Table S2.4:</b> Haplotype diversity amongst mainland Australian and Tasmanian species by proportion of haplotypes per individual.	174
<b>Table S3.1:</b> Samples from Hawkes Bay New Zealand, mainland Australia and Tasmania; geographic location, sex, coat colour; nDNA population and mtDNA haplotype which they fall within in this study.	176
<b>Table S4.1:</b> Metadata for published mtDNA sequences and tissue samples used for phylogenetic analysis.	183
<b>Table S4.2:</b> Threshold analysis for 16s marker <i>16sMam</i> .	190
<b>Table S4.3:</b> The number of correct species identifications observed from the <i>bestCloseMatch</i> analysis for the <i>16sMam</i> marker using three genetic distance thresholds.	190
<b>Table S4.4:</b> Species identifications, and sequences that could not be identified, observed from the <i>threshID</i> analysis for the <i>16sMam</i> marker.	191
<b>Table S4.5:</b> Threshold analysis for 12s marker <i>12sV5</i> .	191
<b>Table S4.6:</b> The number of correct species identifications observed from the <i>bestCloseMatch</i> analysis for the <i>12sV5</i> marker using three genetic distance thresholds.	192
<b>Table S4.7:</b> The number of correct, incorrect and ambiguous species identifications, and sequences that could not be identified, observed from the <i>threshID</i> analysis for the <i>12sV5</i> marker.	192

<b>Table S5.1:</b> All scat samples which begun the metabarcoding process, CT-values and 12s MID-tag associated with each library for DNA metabarcoding.....	193
<b>Table S5.2:</b> All scat samples which begun the metabarcoding process, CT-values and 16s MID-tag associated with each library for DNA metabarcoding.....	205
<b>Table S5.3:</b> Predator detections from predator test and corresponding predator detection from DNA metabarcoding.....	218
<b>Table S5.4:</b> Number of prey species detections in predator scats for each survey period (NE – north east Tasmania, NC – north central Tasmania).....	228

## LIST OF FIGURES

<b>Figure 1.1:</b> Sahul, or Greater Australia, the landmass which encompassed Tasmania, Australian mainland and Papua New Guinea during the Pleistocene.....	4
<b>Figure 1.2:</b> The isolation of Tasmania from mainland Australia.....	5
<b>Figure 2.1:</b> The potential divergence scenarios of lineages from Tasmania and the adjacent Australian mainland.....	18
<b>Figure 2.2:</b> General framework for the detection of an invasive native species, and the application of this framework to an Australian marsupial, <i>Petaurus breviceps</i> .....	19
<b>Figure 2.3:</b> Historical species occurrences and observational records of <i>Petaurus breviceps</i> across Tasmania from 1840 to present day.....	24
<b>Figure 2.4:</b> Median joining network for concatenated ND2 and ND4 mitochondrial genes and the $\omega$ -globin nuclear gene for the sugar glider across the species geographic range.....	26
<b>Figure 2.5:</b> Schematic of sugar glider sample locations across the geographic distribution of combined with the concatenated ND2 and ND4 mitochondrial genes and the nuclear $\omega$ -globin gene across the species geographic distribution (clades represented only).....	27
<b>Figure 3.1:</b> Schematic of collection locations of samples used in this study from (a) Australia and Tasmania and (b) Hawkes Bay, New Zealand.....	41
<b>Figure 3.2:</b> PCoA of SNP genotypes from Australian brushtail possums sampled from mainland Australia (orange) and Tasmania (green).....	44
<b>Figure 3.3:</b> PCoA of SNP genotypes from possums sampled from the Hawkes Bay area of New Zealand.....	45
<b>Figure 3.4:</b> Structure plot of Hawkes Bay, NZ, brushtail possums.....	46
<b>Figure 3.5:</b> Pairwise comparisons of fixed differences, or fixed alleles within a population, between individual possums in the Hawkes Bay area of New Zealand.....	48
<b>Figure 3.6:</b> PCoA of SNP genotypes from brushtail possums sampled from the Hawkes Bay region of New Zealand (n=253), mainland Australia (n=19) and Tasmania (n=11).....	50
<b>Figure 3.7:</b> Spatial map showing the likely ancestry of possums in the Hawkes Bay region of New Zealand, based on SNP genotype data.....	52
<b>Figure 4.1:</b> Phylogenetic tree at the level of Class, generated for the COI <i>uni-minibar</i> amplicon using R package PrimerTree.....	72
<b>Figure 4.2:</b> Phylogenetic trees at the level of Class, generated for the 16s amplicons (A) <i>L2513 – H2714</i> and (B) <i>16sMamF - 16sMamR</i> using R package PrimerTree.....	73

<b>Figure 4.3:</b> Phylogenetic tree at the level of Class, generated for the 12s amplicon <i>12sV5</i> using R package PrimerTree.....	74
<b>Figure 4.4:</b> Evaluation of the conservation of primer binding sites across OTUs for the seven marsupial orders at the 16s mitochondrial gene.....	76
<b>Figure 4.5:</b> Evaluation of the conservation of primer binding sites across OTU for the seven marsupial orders at the 12s mitochondrial gene.....	77
<b>Figure 4.6:</b> Primer evaluation of OTU for the seven marsupial orders at three mitochondrial genes: 12s ( <i>12sV5</i> ), 16s ( <i>16sMam</i> ) and COI ( <i>uni-minibar</i> ).....	78
<b>Figure 4.7:</b> Maximum likelihood tree for Tasmanian Diprotodontia at the mitochondrial gene 16s.....	82
<b>Figure 4.8:</b> Maximum likelihood tree for Tasmanian Diprotodontia at the mitochondrial gene 12s.....	83
<b>Figure 5.1:</b> Survey units from which scats were collected in north central and north-eastern Tasmania.....	96
<b>Figure 5.2:</b> Predator test of 2008 scat DNA, amplified at 16s.....	98
<b>Figure 5.3:</b> Stacked bar plots showing the proportion of scats assigned to each of the three predator groups in the two survey areas.....	105
<b>Figure 5.4:</b> Ternary plot showing the proportion of prey detections associated with each of three types of predator.....	107
<b>Figure 5.5:</b> Principal component analysis showing differences in diet composition among three marsupial predators in the north of Tasmania.....	108
<b>Figure 5.6:</b> Whisker plot generated in the R package JAGS showing the effect of devil presence on prey diet diversity per survey unit in a) cat scats and b) quoll scats.....	109
<b>Figure 5.7:</b> Relative proportions of different prey taxa detected from devil, quoll and cat scats in the north east and north central survey areas.....	111
<b>Figure S2.1:</b> Minimum joining network for mitochondrial ND4 gene.....	147
<b>Figure S2.2:</b> Maximum likelihood tree using unique sequences only, for the mitochondrial gene ND4.....	148
<b>Figure S2.3:</b> Minimum joining network for nuclear gene $\omega$ -globin.....	149
<b>Figure S2.4:</b> Maximum likelihood tree using unique sequences only, for the nuclear gene $\omega$ -globin.....	150

<b>Figure S2.5:</b> Minimum joining network for mitochondrial ND2 gene.....	151
<b>Figure S2.6:</b> Maximum likelihood tree using unique sequences only, for the mitochondrial gene ND2.....	152
<b>Figure S2.7:</b> Mismatch analysis for Tasmanian individuals and individuals from the closest haplotypes clustered around the Tasmanian haplotype.....	153
<b>Figure S2.8:</b> DIYABC scenario showing the predicted split in populations of <i>P. breviceps</i> ; (Figure 2: hypothesis a or c).....	154
<b>Figure S2.9:</b> DIYABC model check.....	155
<b>Figure S2.10:</b> DIYABC analysis showing estimated divergence time for the Aus1 clade population.....	155
<b>Figure S2.11:</b> Haplotype diversity for Tasmanian recent founder, trans-Tasman native and a Tasmanian endemic species.....	156
<b>Figure S5.1:</b> Total cat diet for north east (2008).....	231
<b>Figure S5.2:</b> Total cat diet in the north central survey area (2010).....	232
<b>Figure S5.3:</b> Total cat diet in the north east survey area (2008).....	233
<b>Figure S5.4:</b> Total <i>Dasyurus spp.</i> diet in the north east survey area (2008).....	234
<b>Figure S5.5:</b> Total <i>Dasyurus spp.</i> diet in the north central survey area (2010).....	235
<b>Figure S5.6:</b> Total <i>Dasyurus spp.</i> diet in the north east survey area (2014).....	236
<b>Figure S5.7:</b> Total devil diet in the north east survey area (2008).....	237
<b>Figure S5.8:</b> Total devil diet in the north central survey area (2010).....	238
<b>Figure S5.9:</b> Total devil diet in the north east survey area (2014).....	239