

Species from faeces: metabarcoding to detect vertebrate prey from predator scats

Anna J. MacDonald¹, Dianne Gleeson¹, Michael Bunce² and Stephen D. Sarre¹

¹ Institute for Applied Ecology, University of Canberra, Canberra, ACT, Australia
² School of Veterinary and Life Sciences, Murdoch University, Perth, Western Australia, Australia

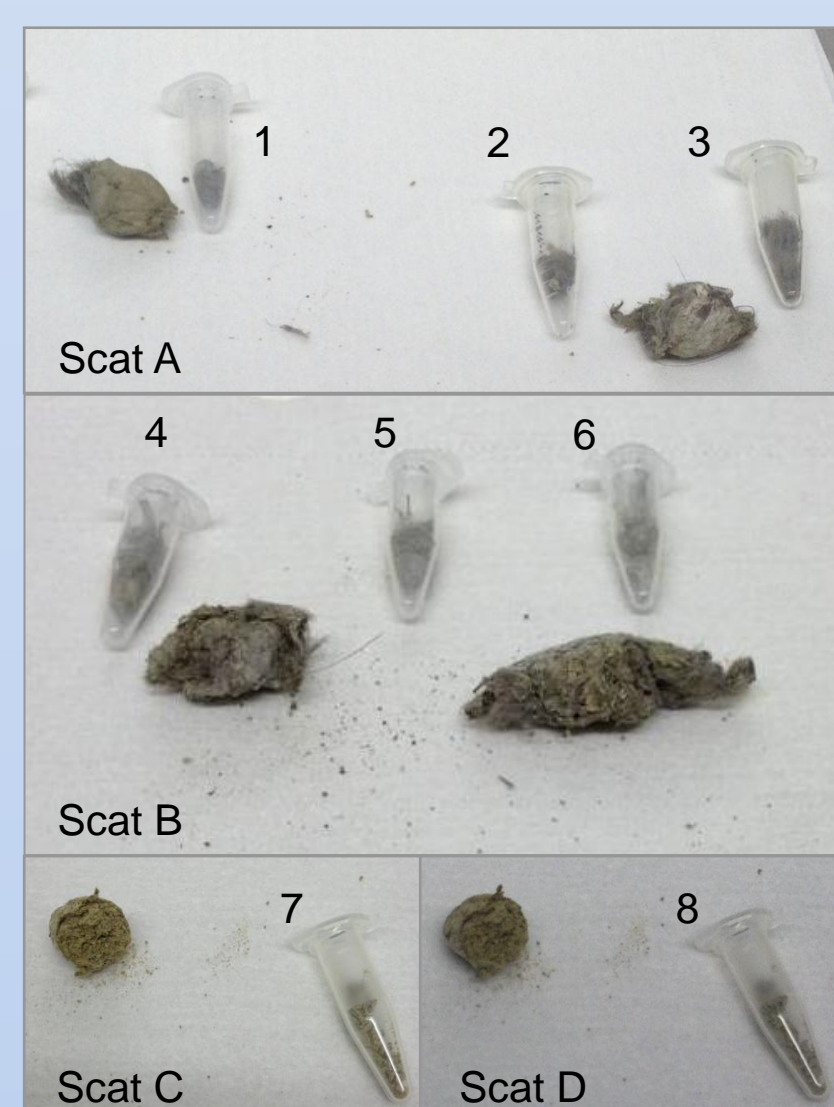
BACKGROUND:

Native wildlife across Australia are vulnerable to predation by introduced dogs, cats and foxes. The recent introduction of foxes to Tasmania poses a serious threat to many species, particularly small to medium sized mammals, that have almost completely disappeared from mainland Australia. Concurrently, the Tasmanian devil, the largest native predator, is suffering a rapid decline. Both events are expected to significantly alter predator-prey dynamics in Tasmania. DNA from predator scats (faeces) provides valuable information on the diets of, and threats to, rare and cryptic species. We will use NGS and a metabarcoding approach to simultaneously identify both predator and prey species from scat DNA, providing critical information on predator diets, prey species distributions and predator-prey interactions.

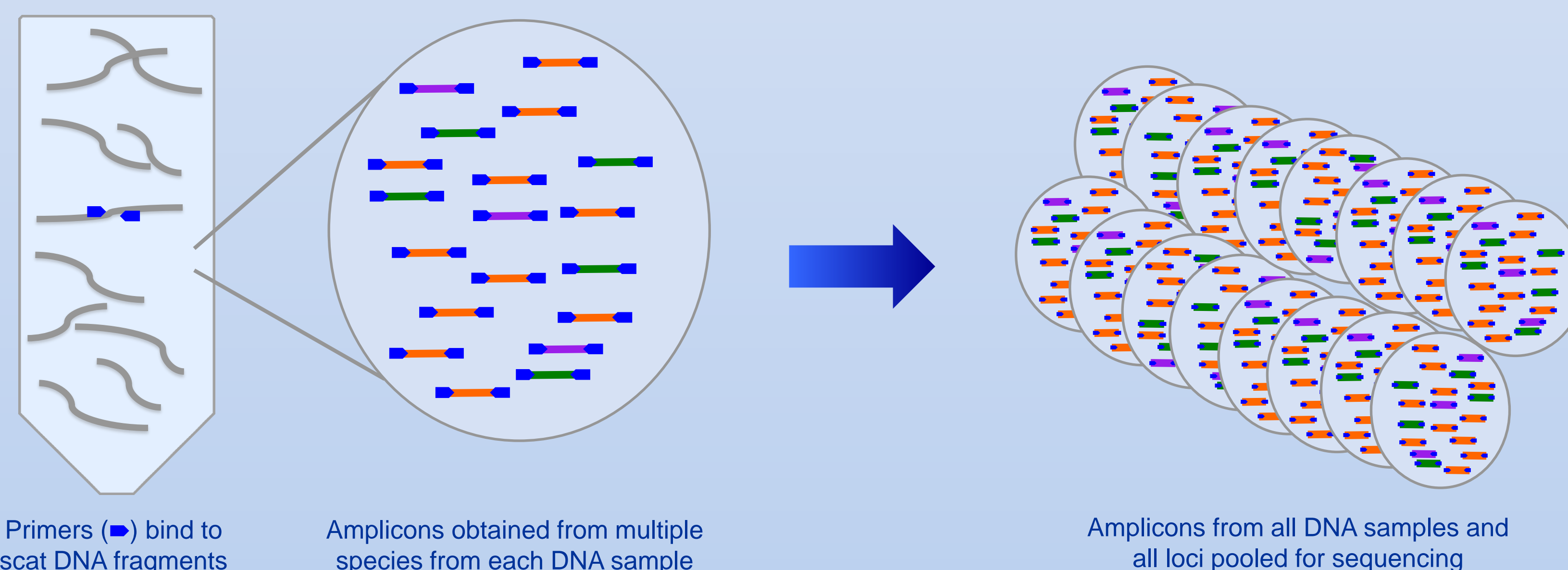
PILOT STUDY:

DNA Extraction:

DNA was extracted from four predator scats, collected in Tasmania between 2009 and 2011, using the ChargeSwitch Forensic DNA Purification Kit (Invitrogen). For scats A and B, three different DNA extractions were prepared from different portions of the scat.

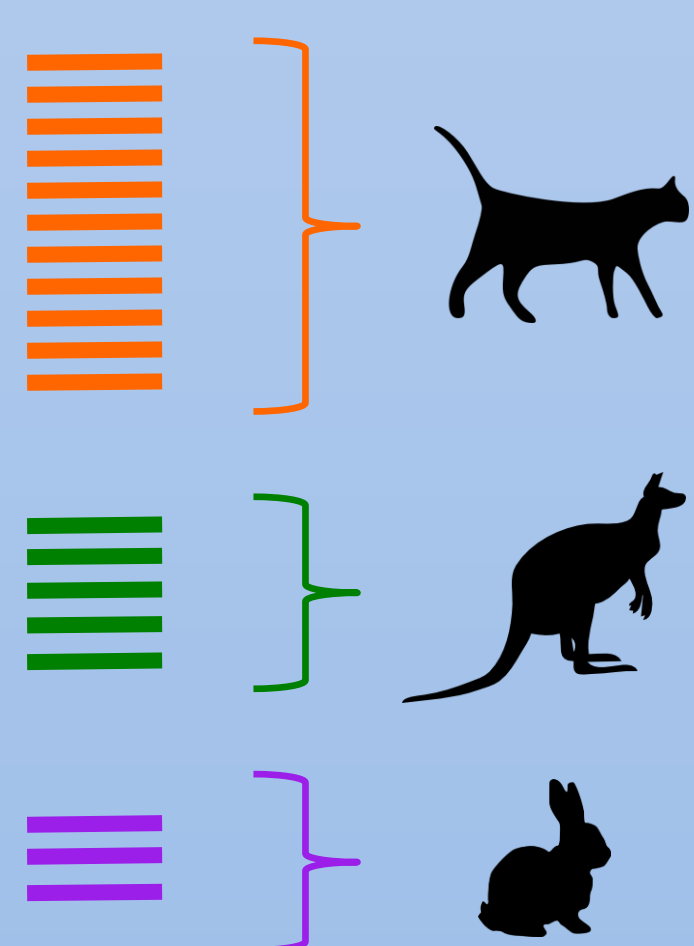


PCR and Sequencing: Scat DNA was amplified using two primer sets targeting mammalian 16S and vertebrate 12S sequences. Amplicons (<200bp) were sequenced on the GS Junior platform.



Data Analysis: Sequence reads were assigned to taxonomic groups using MEGAN, following BLAST against GenBank.

Results: DNA from at least two different species was detected from each scat. Results were generally consistent between 12S and 16S amplicons: notably the *Lampropholis* skink DNA was not amplified using mammalian 16S primers. There was some variation among replicates for scats with multiple DNA extractions. Table shows species detected from each sample (number of sequence reads that passed QC in parentheses).



Genus	Common name	Scat A extraction 1	Scat A extraction 2	Scat A extraction 3	Scat B extraction 1	Scat B extraction 2	Scat B extraction 3	Scat C	Scat D
Felis	Cat	12S (609) 16S (225)	12S (455) 16S (197)	12S (645) 16S (228)	12S (511) 16S (164)	12S (390) 16S (117)	12S (433) 16S (204)	12S (254) 16S (220)	12S (407) 16S (147)
Oryctolagus	Rabbit								12S (9) 16S (7)
Mus	Mouse	12S (27) 16S (6)	12S (30) 16S (15)	12S (23) 16S (2)	12S (40) 16S (14)	12S (3) 16S (4)	12S (1) 16S (4)		
Rattus	Rat	12S (4) 16S (2)	12S (15) 16S (4)	12S (4) 16S (2)	12S (1)		12S (7)		
Pseudocheirus	Ringtail possum				12S (8) 16S (3)	12S (46) 16S (16)	12S (4) 16S (2)		
Thylogale	Tasmanian pademelon							12S (53) 16S (45)*	
Lampropholis	Skink				12S (6)				

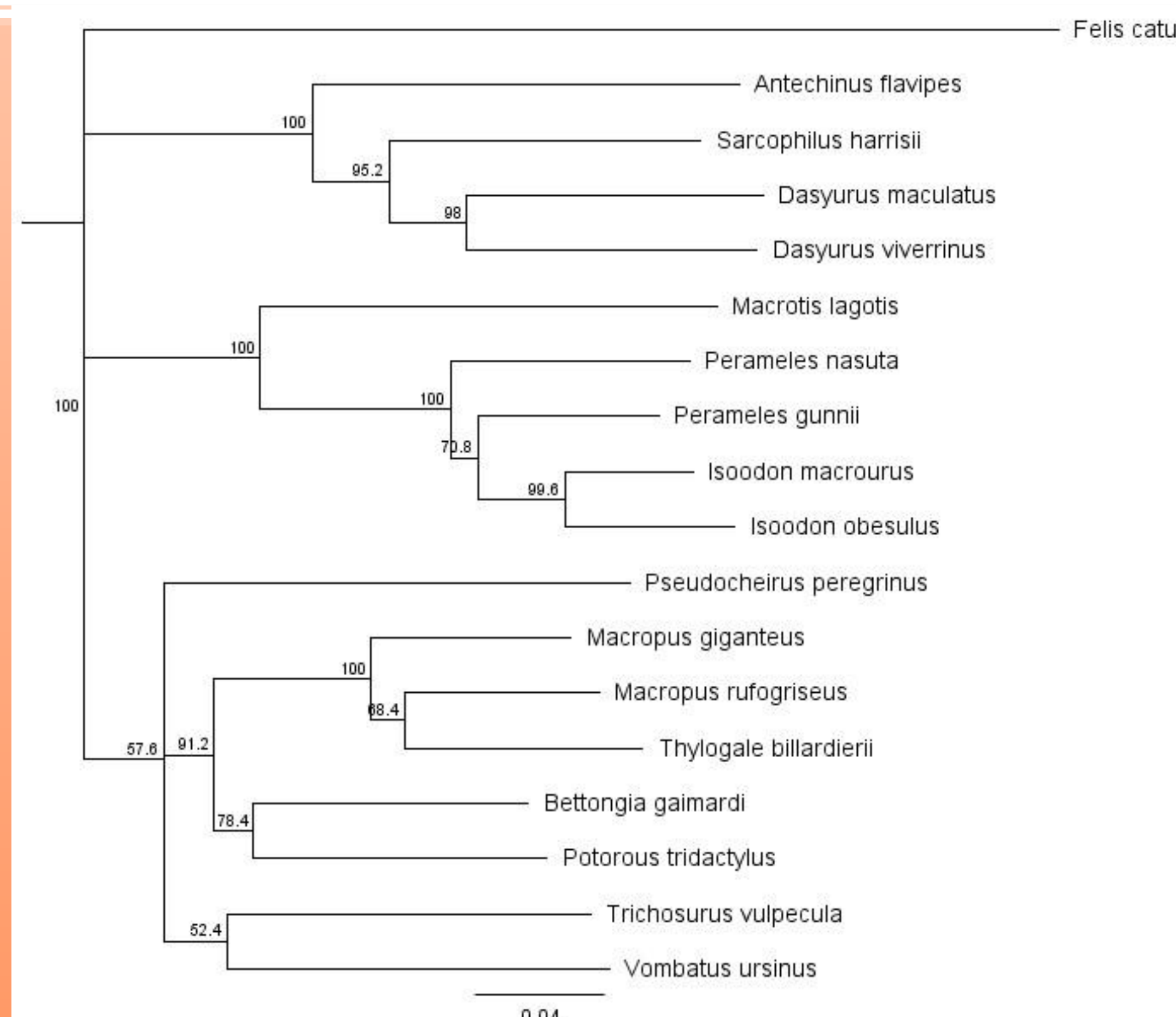
* NB 16S sequence data for Thylogale are not yet publicly available, closest GenBank hits are to several other macropodids.

Future work: We will now apply these methods to analyse hundreds of predator scats collected from across Tasmania since 2008. We aim to determine predator-prey interactions for native and introduced species and to identify species distributions for a range of cryptic taxa. Further sample collection planned for 2014 will enable comparison of data over 4-5 years for some geographic locations, encompassing significant devil population declines.

A DNA REFERENCE DATABASE FOR AUSTRALIAN VERTEBRATES:

Our initial results highlight numerous gaps in public DNA sequence databases. To improve our ability to interpret results, we are developing custom DNA sequence databases for Australian terrestrial vertebrates at several mitochondrial genes. As well as 12S and 16S, we will target additional genes to improve our resolution for certain taxa, e.g. the ND2 gene may be especially useful for identification of and discrimination among marsupial species.

We are keen to talk to potential collaborators who may be able to contribute reference samples or DNA sequences



Tree based on 784 bp of the ND2 gene: ND2 provides good resolution for marsupials.