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Wildlife Research

Supplementary Material

Evaluation of genetic markers for the metabarcoding of Australian marsupials from predator scats

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Supplementary Tables

Table S1: Sample ID, NCBI GenBank accession numbers, location details and references for published mtDNA sequences and tissue samples used for phylogenetic analysis and including samples collected within this study(*) (ABTC: South Australia Museum, AM M: Australia Museum, ANWC M: Australian National Wildlife Collection, U: Museum and Art Gallery of Northern Territory (MAGNT), A: Tasmanian Museum and Art Gallery (TMAG), QVM: Queen Victoria Museum and Art Gallery (QVMAG), AA/UC: University of Canberra Tissue Collection). ° WA – Western Australia; Vic – Victoria; NSW – New South Wales; SA – South Australia; ACT – Australian Capital Territory; NLD – No location data provided.

SPECIES	Sample #	12S rRNA	16S rRNA	Location	Reference
<i>Bettongia gaimardi</i>		AY245619		Tasmania	[1]
<i>Bettongia gaimardi</i>	A4708	PP923472		Tasmania	*
<i>Bettongia gaimardi</i>	AA15140	PP923476	PP923427	Tasmania	*
<i>Bettongia gaimardi</i>	AA15141	PP923477	PP923428	Tasmania	*
<i>Bettongia gaimardi</i>	AA15142	PP923478	PP923429	Tasmania	*
<i>Bettongia gaimardi</i>	AA15145		PP923430	Tasmania	*
<i>Bettongia gaimardi</i>	AA15146	PP923479		Tasmania	*
<i>Bettongia gaimardi</i>	Bga001	PP923499		Tasmania	*
<i>Bettongia gaimardi</i>	Bga006	PP923500	PP923431	Tasmania	*
<i>Bettongia gaimardi</i>	Bga008	PP923501	PP923432	Tasmania	*
<i>Bettongia gaimardi</i>	Bga009	PP923502		Tasmania	*
<i>Bettongia gaimardi</i>	Bga012	PP923503	PP923433	Tasmania	*
<i>Bettongia gaimardi</i>	UC1564	PP923533		Tasmania	*
<i>Bettongia gaimardi</i>	UC1585	PP923538		Tasmania	*
<i>Bettongia gaimardi</i>	UC1589	PP923539		Tasmania	*
<i>Bettongia gaimardi</i>	UC1592	PP923540		Tasmania	*
<i>Bettongia gaimardi</i>	UC1593	PP923541		Tasmania	*
<i>Bettongia gaimardi</i>	UC1594	PP923542		Tasmania	*
<i>Bettongia gaimardi</i>	UC1595	PP923543	PP923434	Tasmania	*
<i>Bettongia gaimardi</i>	UC1596	PP923544	PP923435	Tasmania	*

<i>Bettongia gaimardi</i>	UC1597	PP923545	PP923436	Tasmania	*
<i>Bettongia gaimardi</i>	UC1598	PP923546	PP923437	Tasmania	*
<i>Bettongia gaimardi</i>	UC1599	PP923547	PP923438	Tasmania	*
<i>Bettongia gaimardi</i>	UC1600	PP923548	PP923439	Tasmania	*
<i>Bettongia gaimardi</i>	UC1646	PP923554		Tasmania	*
<i>Bettongia gaimardi</i>	UC1648	PP923555		Tasmania	*
<i>Bettongia gaimardi</i>	UC1649	PP923556		Tasmania	*
<i>Bettongia gaimardi</i>	UC1650	PP923557		Tasmania	*
<i>Bettongia gaimardi</i>	UC1651	PP923558		Tasmania	*
<i>Bettongia gaimardi</i>	UC1652	PP923559		Tasmania	*
<i>Bettongia gaimardi</i>	UC1653	PP923560		Tasmania	*
<i>Bettongia gaimardi</i>	UC1654	PP923561		Tasmania	*
<i>Bettongia lesueur</i>		AY245620		NLD	[1]
<i>Bettongia lesueur</i>		KJ868101		WA	[2]
<i>Bettongia penicillata</i>		AF027998		NLD	[3]
<i>Bettongia penicillata</i>		AY245612		WA	[1]
<i>Bettongia penicillata</i>	ABTC26751	KJ868102		WA	[2]
<i>Cercartetus nanus</i>		JN255346			[4]
<i>Notamacropus agilis</i>		AF027986			[3]
<i>Notamacropus eugenii</i>		KJ868119	KJ868119	SA	[2]
<i>Notamacropus eugenii</i>		AY012092			[5]
<i>Notamacropus eugenii</i>		AY245606	JN003391	ACT	[1,6]
<i>Macropus giganteus</i>		LK995454	LK995454	ACT	[7]
<i>Macropus giganteus</i>		NC027424	NC027424	ACT	[7]
<i>Macropus giganteus</i>		AF187885		NLD	[8]
<i>Macropus giganteus</i>		AY245609		NLD	[1]
<i>Macropus giganteus</i>		X86941		NLD	[9]
<i>Notamacropus parryi</i>		AY245610		NLD	[1]
<i>Notamacropus parryi</i>		AF187887		NLD	[8]
<i>Notamacropus rufogriseus</i>		AY245607		NLD	[1]
<i>Notamacropus rufogriseus</i>	A3353	PP923470	PP923440	Tasmania	*
<i>Notamacropus rufogriseus</i>	AA15164	PP923488	PP923441	Tasmania	*
<i>Notamacropus rufogriseus</i>	AA15165		PP923442	Tasmania	*
<i>Notamacropus rufogriseus</i>	AA15166	PP923489		Tasmania	*

<i>Notamacropus rufogriseus</i>	AA15167	PP923490	PP923443	Tasmania	*
<i>Notamacropus rufogriseus</i>	AA15168	PP923491		Tasmania	*
<i>Notamacropus rufogriseus</i>	AA15169		PP923444	Tasmania	*
<i>Notamacropus rufogriseus</i>	ABTC83648	KJ868122	KJ868122	Tasmania	[2]
<i>Notamacropus rufogriseus</i>	M29356	JN003394	JN003395	NSW	[6]
<i>Notamacropus rufogriseus</i>	UC1212	PP923513		Tasmania	*
<i>Notamacropus rufogriseus</i>	UC1221	PP923515	PP923445	Tasmania	*
<i>Notamacropus rufogriseus</i>	UC1234	PP923522	PP923446	Tasmania	*
<i>Notamacropus rufogriseus</i>	UC1235		PP923447	Tasmania	*
<i>Notamacropus rufogriseus</i>	UC1237	PP923523	PP923448	Tasmania	*
<i>Notamacropus rufogriseus</i>	UC1238		PP923449	Tasmania	*
<i>Petaurus breviceps</i>		AB241055	AB241055	NLD	[10]
<i>Petaurus breviceps</i>		AY228380	AY228380	NLD	[11]
<i>Petaurus breviceps</i>		NC008135	NC008135	NLD	[10]
<i>Petaurus breviceps</i>	A3410	PP923471		Tasmania	*
<i>Petaurus breviceps</i>	AA61732	PP923492	PP923450	Tasmania	*
<i>Petaurus breviceps</i>	AA61735	PP923493	PP923451	Tasmania	*
<i>Petaurus breviceps</i>	AA61737	PP923494		Tasmania	*
<i>Petaurus breviceps</i>	AA61738	PP923495		Tasmania	*
<i>Petaurus breviceps</i>	AA61740	PP923496		Tasmania	*
<i>Petaurus breviceps</i>	AA61741		PP923452	Tasmania	*
<i>Petaurus breviceps</i>	AA61744	PP923497		Tasmania	*
<i>Petaurus breviceps</i>	AA61746	PP923498		Tasmania	*
<i>Petaurus breviceps</i>	UC1736	PP923568		Tasmania	*
<i>Phascolarctos cinereus</i>		NC008133	NC008133	NLD	[10]
<i>Phascolarctos cinereus</i>		U61076	AF166344	NLD	[12,13]
<i>Phascolarctos cinereus</i>	98.122	AB241053	AB241053	NLD	[10]
<i>Potorous gilbertii</i>		AY245616		NLD	[1]
<i>Potorous gilbertii</i>		JX104630		WA	[14]
<i>Potorous longipes</i>		AY245624		Vic	[1]
<i>Potorous longipes</i>		AY245614		NLD	[1]
<i>Potorous longipes</i>			KJ868148	Vic	[2]
<i>Potorous longipes</i>			AF028000	Vic	[3]
<i>Potorous longipes</i>		JX104631		Vic	[14]

<i>Potorous tridactylus</i>		AY245617		NLD	[1]
<i>Potorous tridactylus</i>			AJ639873	NLD	[15]
<i>Potorous tridactylus</i>	A3864	.		Tasmania	*
<i>Potorous tridactylus</i>	AA15135	PP923473		Tasmania	*
<i>Potorous tridactylus</i>	AA15136		PP923453	Tasmania	*
<i>Potorous tridactylus</i>	AA15139	PP923475		Tasmania	*
<i>Potorous tridactylus</i>	M24814	PP923504		Tasmania	*
<i>Potorous tridactylus</i>	UC1571	.		Tasmania	*
<i>Potorous tridactylus</i>	UC1573	PP923534		Tasmania	*
<i>Potorous tridactylus</i>	UC1574	.		Tasmania	*
<i>Potorous tridactylus</i>	UC1575	PP923535		Tasmania	*
<i>Potorous tridactylus</i>	UC1576	PP923536		Tasmania	*
<i>Potorous tridactylus</i>	UC1577	PP923537		Tasmania	*
<i>Potorous tridactylus</i>	UC1578	.		Tasmania	*
<i>Potorous tridactylus apicalis</i>		JX104628		Tasmania	
<i>Potorous tridactylus apicalis</i>		JX104627		Tasmania	
<i>Potorous tridactylus apicalis</i>		JX104629		Tasmania	[14]
<i>Potorous tridactylus tridactylus</i>	(A)M39201	JX104621		NSW	[14]
<i>Potorous tridactylus tridactylus</i>	(A)M41008	JX104622		NSW	[14]
<i>Potorous tridactylus tridactylus</i>	W30	JX104619		QLD	[14]
<i>Potorous tridactylus tridactylus</i>	W34	JX104620		QLD	[14]
<i>Pseudocheirus peregrinus</i>		AJ639870	AJ639870	NLD	[15]
<i>Pseudocheirus peregrinus</i>	AA15161	PP923487		Tasmania	*
<i>Pseudocheirus peregrinus</i>	UC1640	PP923553		Tasmania	*
<i>Pseudocheirus peregrinus</i>	UC1737	PP923567	PP923454	Tasmania	*
<i>Thylodale billardierii</i>	ABTC83755		KJ868162	Tasmania	[2]
<i>Thylogale billardierii</i>		HQ283961		KI-Tas	[16]

<i>Thylogale billardierii</i>		HQ283960		FI-Tas	[16]
<i>Thylogale billardierii</i>	AA15146	PP923479		Tasmania	*
<i>Thylogale billardierii</i>	AA15147	PP923480	PP923455	Tasmania	*
<i>Thylogale billardierii</i>	AA15149	PP923481	PP923456	Tasmania	*
<i>Thylogale billardierii</i>	AA15150	PP923482		Tasmania	*
<i>Thylogale billardierii</i>	AA15151	PP923483		Tasmania	*
<i>Thylogale billardierii</i>	UC1200	PP923507		Tasmania	*
<i>Thylogale billardierii</i>	UC1208	PP923509	PP923457	Tasmania	*
<i>Thylogale billardierii</i>	UC1209	PP923510		Tasmania	*
<i>Thylogale billardierii</i>	UC1210	PP923511	PP923458	Tasmania	*
<i>Thylogale billardierii</i>	UC1216		PP923459	Tasmania	*
<i>Thylogale billardierii</i>	UC1219	PP923514	PP923460	Tasmania	*
<i>Thylogale billardierii</i>	UC1223	PP923517		Tasmania	*
<i>Thylogale billardierii</i>	UC1224	PP923518	PP923461	Tasmania	*
<i>Thylogale billardierii</i>	UC1232	PP923521		Tasmania	*
<i>Thylogale billardierii</i>	UC1239	PP923524		Tasmania	*
<i>Thylogale billardierii</i>	UC1241	PP923525	PP923462	Tasmania	*
<i>Thylogale billardierii</i>	UC1242	PP923526	PP923463	Tasmania	*
<i>Thylogale billardierii</i>	UC1243		PP923464	Tasmania	*
<i>Thylogale billardierii</i>	UC1244	PP923527		Tasmania	*
<i>Thylogale billardierii</i>	UC1246	PP923528	PP923465	Tasmania	*
<i>Thylogale billardierii</i>	UC1249	PP923529	PP923466	Tasmania	*
<i>Thylogale billardierii</i>	UC1463	PP923531		Tasmania	*
<i>Thylogale billardierii</i>	UC1464	PP923532		Tasmania	*
<i>Thylogale billardierii</i>	UC1606		PP923467	Tasmania	*
<i>Thylogale thetis</i>		HQ283958		QLD	[16]
<i>Thylogale thetis</i>		HQ283959		NSW	[16]
<i>Trichosurus vulpecula</i>			NC003039	NZ	[17]
<i>Trichosurus vulpecula</i>			AF357238	NZ	[17]
<i>Trichosurus vulpecula</i>		AF031823	AF187888	QLD	[8]
<i>Trichosurus vulpecula</i>	AA15153	PP923484		Tasmania	*
<i>Trichosurus vulpecula</i>	AA15155	PP923485		Tasmania	*
<i>Trichosurus vulpecula</i>	AA15157	PP923486		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1211	PP923512		Tasmania	*

<i>Trichosurus vulpecula</i>	UC1222	PP923516		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1228	PP923519		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1229	PP923520		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1252	PP923530		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1601	PP923549		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1604	PP923550		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1608	PP923551		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1613	PP923552		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1671		PP923468	Tasmania	*
<i>Trichosurus vulpecula</i>	UC1672	UC1672		Tasmania	*
<i>Trichosurus vulpecula</i>	UC1673		PP923469	Tasmania	*
<i>Vombatus ursinus</i>		NC 003322		NLD	[18]
<i>Vombatus ursinus</i>		AJ304826		NLD	[18]
<i>Vombatus ursinus</i>		U61078		NLD	[12]
<i>Sister group for phylogenetic analysis</i>					
<i>Dasyurus albopunctatus</i>			AF166349	NLD	[13]
<i>Dasyurus albopunctatus</i>		AF009890		NLD	[19]
<i>Dasyurus geoffroii</i>			AY528921	NLD	[20]
<i>Dasyurus geoffroii</i>		KJ780027		WA	[21]
<i>Dasyurus hallucatus</i>			AY528917	NLD	[20]
<i>Dasyurus hallucatus</i>		U87400		NLD	[19]
<i>Dasyurus maculatus</i>			AY528918	NLD	[20]
<i>Dasyurus maculatus</i>		KJ780028		Tasmania	[21]
<i>Dasyurus viverrinus</i>			AY528920	NLD	[20]
<i>Dasyurus viverrinus</i>		U87401		NLD	[19]

References

- Burk A, Springer MS (2000). Intergeneric relationships among macropodoidea (Metatheria : Diprotodontia) and the chronicle of kangaroo evolution. *Journal of Mammalian Evolution* 7, 213–237.

- Burk A, Westerman M, Kao DJ, Kavanagh JR, Springer MS (1999). An Analysis of Marsupial Interordinal Relationships Based on 12S rRNA, tRNA Valine, 16S rRNA, and Cytochrome b Sequences. *Journal of Mammalian Evolution* **6**, 317–334.
- Burk A, Westerman M, Springer M (1998). The phylogenetic position of the Musky rat-kangaroo and the evolution of bipedal hopping in kangaroos (Macropodidae: Diprotodontia). *Systematic Biology Biol.* **47**, 457–474.
- Dotl WG, McComish BJ, Nilsson M a., Gibb GC, Penny D, Phillips MJ (2014). The complete mitochondrial genome of the eastern grey kangaroo (*Macropus giganteus*). *Mitochondrial DNA* **1736**, 1–2. doi:10.3109/19401736.2014.947583
- Douzery E, Catzeflis FM (1995). Molecular evolution in the mitochondrial 12s rRNA in Ungulata (Mammalia). *Journal of molecular Evolution* **41**, 622–636.
- Frankham GJ, Handasyde KA, Eldridge MDB (2012). Novel insights into the phylogenetic relationships of the endangered marsupial genus Potorous. *Molecular Phylogenetics and Evolution* **64**, 592–602. doi:10.1016/j.ympev.2012.05.013
- Janke A, Magnell O, Wieczorek G, Westerman M, Arnason U (2002). Phylogenetic Analysis of 18S rRNA and the Mitochondrial Genomes of the Wombat, *Vombatus ursinus*, and the Spiny Anteater, *Tachyglossus aculeatus*: Increased Support for the Marsupionta Hypothesis. *Journal of Molecular Evolution* **54**, 71–80. doi:10.1007/s00239-001-0019-8
- Kavanagh J, Burk-Herrick A, Westerman M, Springer MS (2004). Relationships among families of Diprotodontia (Marsupialia) and the phylogenetic position of the autapomorphic honey possum (*Tarsipes rostratus*). *Journal of Mammalian Evolution* **11**, 207–222.
- Krajewski C, Moyer GR, Sipiorski JT, Fain MG, Westerman M (2004). Molecular systematics of the enigmatic ‘phascolosoricine’ marsupials of New Guinea. *Australian Journal of Zoology* **52**, 389. doi:10.1071/ZO04020
- Krajewski C, Young J, Buckley L, Woolley PA, Westerman (1997). Reconstructing the evolutionary radiation of dasyurine marsupials with cytochrome b, 12S rRNA, and protamine P1 gene trees. *Journal of Mammalian Evolution* **150**, 85–115.
- Macqueen P, Seddon J, Austin J, Hamilton S, Goldizen A (2010). Phylogenetics of the pademelons (Macropodidae: Thylogale) and historical biogeography of the Australo-Papuan region. *Molecular Phylogenetics and Evolution* **57**, 1134–1148. doi:10.1016/j.ympev.2010.08.010
- Mitchell KJ, Pratt RC, Watson LN, Gibb GC, Llamas B, Kasper M, Edson J, Hopwood B, Male D, Armstrong KN, Meyer M, Hofreiter M, Austin J, Donnellan SC, Lee MSY, Phillips MJ, Cooper A (2014). Molecular phylogeny, biogeography, and habitat preference evolution of marsupials. *Molecular Biology and Evolution* **31**, 2322–2330. doi:10.1093/molbev/msu176

- Munemasa M, Nikaido M, Donnellan S, Austin CC, Okada N, Hasegawa M (2006). Phylogenetic analysis of diprotodontian marsupials based on complete mitochondrial genomes. *Genes & Genetic Systems* **81**, 181–191.
- Murphy WJ, Eizirik E, Johnson WE, Zhang YP, Ryder OA, O'Brien SJ (2001). Molecular phylogenetics and the origins of placental mammals. *Nature* **409**, 614–618.
- Nilsson MA, Arnason U, Spencer PBS, Janke A (2004). Marsupial relationships and a timeline for marsupial radiation in South Gondwana. *Gene* **340**, 189–96. doi:10.1016/j.gene.2004.07.040
- Phillips MJ, Haouchar D, Pratt RC, Gibb GC, Bunce M (2013). Inferring kangaroo phylogeny from incongruent nuclear and mitochondrial genes. *PloS one* **8**, e57745–e57745. doi:10.1371/journal.pone.0057745
- Phillips MJ, Lin YH, Harrison GL, Penny D (2001). Mitochondrial genomes of a bandicoot and a brushtail possum confirm the monophyly of australidelphian marsupials. *Proceedings. Biological sciences / The Royal Society* **268**, 1533–8. doi:10.1098/rspb.2001.1677
- Springer MS, Douzery E (1996). Secondary structure and patterns of evolution among mammalian mitochondrial 12S rRNA molecules. *Journal of molecular evolution* **43**, 357–373. doi:10.1007/BF02339010
- Westerman M, Kear BP, Aplin K, Meredith RW, Emerling C, Springer MS (2012). Phylogenetic relationships of living and recently extinct bandicoots based on nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **62**, 97–108. doi:10.1016/j.ympev.2011.09.009
- Westerman M, Loke S, Springer MS (2004). Molecular phylogenetic relationships of two extinct potoroid marsupials, Potorous platyops and Caloprymnus campestris (Potoroinae: Marsupialia). *Molecular Phylogenetics and Evolution* **31**, 476–485. doi:10.1016/j.ympev.2003.08.006
- Woolley P a., Krajewski C, Westerman M (2015). Phylogenetic relationships within Dasyurus (Dasyuromorphia: Dasyuridae): quoll systematics based on molecular evidence and male characteristics. *Journal of Mammalogy* **96**, 37–46. doi:10.1093/jmammal/gyu028

Table S2: Correct species identification using *bestCloseMatch* for *16sMam* (n = 73), at three thresholds. All incorrect identifications were due to within species diversity.

Genetic distance (%)	Correct identification	Incorrect identification
1%	59	Petaurus_breviceps_AY228380
		Trichosurus_vulpecula_AF187888_QLD
		Trichosurus_vulpecula_UC1671_Tasmania
		Pseudocheirus_peregrinus_AJ639870
		Pseudocheirus_peregrinus_UC1737_Tasmania
3%	63	Petaurus_breviceps_AY228380
5%	63	Petaurus_breviceps_AY228380

Table S3: Species identification using *threshID* for *16sMam* (n = 73) at three different genetic distances. Incorrect identification was identical to the *bestCloseMatch* analysis, however, more individuals were ambiguous for 3% and 5% genetic distance.

Genetic distance (%)	Correct identification	Incorrect identification	Ambiguous identification
1%	59	5	-
3%	49	1	14
5%	47	1	16

Table S4: Correct species identification using *bestCloseMatch* for 12sV5 (n = 199), at three thresholds. All incorrect identifications were due to within species diversity.

Genetic distance (%)	Correct identification	Incorrect identification	Ambiguous
1%	186	Petaurus_breviceps_AY228380	
		Thylogale_billardierii_HQ283961_KI-Tasmania	
		Potorous_tridactylus_AY245617	
		Pseudocheirus_peregrinus_AJ639870	
		Notamacropus_giganteus_Mg232_ACT	
		Notamacropus_parryi_M14000_QLD	
		Trichosurus_vulpecula_UC1673_Tasmania	
		Trichosurus_vulpecula_AF031823_QLD	
		Pseudocheirus_peregrinus_AJ639870	
3%	192	Potorous_tridactylus_AY245617	
5%	193		Potorous_tridactylus_AY245617