Supplementary Information for

Mitogenome of the extinct Desert 'rat-kangaroo' times the adaptation to aridity in macropodoids

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Supplementary tables

Supplementary Table S1. *GenBank* accession numbers for mitogenome and individual mitochondrial (mtDNA) gene sequences used in the phylogenetic analyses. Taxon abbreviations: Arf, *Aepyprymnus rufescens*; Bah*, Bettongia anhydra*; Bgm, *Bettongia gaimardi*; Bls, *Bettongia lesueur*; Bpn, *Bettongia penicillata*; Btp, *Bettongia tropica*; Ccn, *Cercaretus concinus*; Ccp*, *Caloprymnus campestris**; Ddo, *Dendrolagus dorianus*; Dhg, *Dorcopsis hageni*; Dlm; *Dendrolagus lumholtzi*; Dvh, *Dorcopsulus vanheurni*; Hms, *Hypsiprymnodon moschatus*; Lcs, *Lagorchestes conspicillatus*; Lhs, *Lagorchestes hirsutus*; Lfs, *Lagostrophus fasciatus*; Mfg, *Macropus fuliginosus*; Mgg, *Macropus giganteus*; Neg, *Notamacropus eugenii*; Npm, *Notamacropus parma*; Obn, *Osphranter bernardus*; Orb, *Osphranter robustus*; Orf, *Osphranter rufus*; Pak*, *Protemnodon anak**; Pby, *Petrogale brachyotis*; Pgb, *Potorous gilberti*; Ppl*, *Potorous platyops**; Plg, *Potorous longipes*; Pot, *Phalanger orientalis*; Pta, *Potorous tridactylus apicalis*; Ptd, *Potorous tridactylus tridactylus*; Pts, *Potorous tridactylus trisulcatus*; Pxt, *Petrogale xanthopus*; Sbr, *Setonix brachyurus*; Soc*, *Simosthenurus occidentalis**; Tbl, *Thylogale billardierii*; Wbc, *Wallabia bicolor*. *Extinct. [a]Appended partial sequence.

Taxon	mitogenome	Cyt *b*	12S rRNA	16S rRNA	NADH1	COX1/2
			Phalangeridae (outgroup)			
Pot	MN380186	–	–	–	–	–
			Burramyidae (outgroup)			
Ccn	KJ868105	–	–	–	–	–
			Hypsiprymnodontidae			
Hms	KJ868115	–	–	–	–	–
			Potoroidae			
Arf	KJ868095	–	–	–	–	–
Ccp*	MT66337	–	–	–	–	–
Bah*	–	KM974728	–	–	–	–
Bgm	–	AY237244	AY245619	–	KU507321	MK202809
Bls	KJ868101	–	–	–	–	–
Bpn	KJ868102	–	–	–	–	–
Btp	–	AY237236	AY245618	–	JX104579	JX111907
Pgb	–	AY247231	AY245616	–	JX104577	JX111905
Plg	KJ868148	–	–	–	–	–
Ppl*	–	AY237247	AY245621	[a]partial	[a]partial	[a]partial
Pta	NC006524	–	–	–	–	–
Ptd	–	AY237235	JX104620	–	JX104566	JX111894
Pts	–	–	JX104626	–	JX104573	JX111901
			Macropodidae			
Ddo	KJ868110	–	–	–	–	–
Dlm	KJ868111	–	–	–	–	–
Dhg	KJ868112	–	–	–	–	–
Dvh	KJ868113	–	–	–	–	–
Lcs	KY996508	–	–	–	–	–

Taxon (Continued)	mitogenome	Cyt *b*	12S rRNA	16S rRNA	NADH1	COX1/2
Lhs	AB241056	–	–	–	–	–
Lfs	NC008447	–	–	–	–	–
Mfg	KY868120	–	–	–	–	–
Mgg	KY996502	–	–	–	–	–
Neg	KJ868119	–	–	–	–	–
Npm	KY996504	–	–	–	–	–
Obn	KY996505	–	–	–	–	–
Orb	KY996506	–	–	–	–	–
Orf	KY996501	–	–	–	–	–
Pby	KX868141	–	–	–	–	–
Pxt	KX868141	–	–	–	–	–
Pak*	MK190712	–	–	–	–	–
Sbr	KX868156	–	–	–	–	–
Soc*	MK190713	–	–	–	–	–
Tbl	KJ868162	–	–	–	–	–
Wbc	KX868164	–	–	–	–	–

[a]*Potorous platyops** — NADH1 (243 nucleotides):
ATTATTAACCTAcTATTATATATTgTaCCcATTCTaCTAGCAGTaGCTTTtCTAACCCTAGTAGAACGGAAAGTCyTAGGATACATACAATTCCGCAAAGGcCCTA
ACATCGTmGGACCATATGGCCTATTACAACCAATTGCCGATGCCrTwAAACTATTCACAAAAGAACCAyTACGACCyCTwACATCCTCAATCTCAATATTyATT
ATTGCACCCATCCTAGCCTTAACccTaGCcCTA

[a]*Potorous platyops** — COX1 (525 nucleotides):
ATACTATGAGCAcTAGGCTTTATTTTTCTCTTCACTATTGGTGGTTTAACAGGAATTGTTCTAGCCAATTCCTCCCTAGACATCGTTCTCCACGACACCTATTA
TGTAGTAGCACATTTTCACTATGTGTTATCAATAGGAGCCGTATTCGCAATTATAGGAGGATTTGTCCACTGATTCCCCTTATTTACAGGATATACACTCAAC
GATACATGAGCTAAAATCCACTTCTCTGTGATATTTGTAGGAGTTAATCTCACATTCTTCCCACAACACTTCCTAGGCCTCTCTGGCATACCACGACGATATT
CAGATTATCCAGATGCTTATACACTATGAAACGTATTATCATCAATTGGCTCCTTCATTTCACTCACAGCCGTTATCCTAATAGTATTTATTGTCTGAGAAGCC
TTCGCATCAAAACGTGAAGTCTCAACTGTAGAACTAACAACTACCAATATTGAATGACTTTATGGTTGTCCACCACCTTACCATACATTTGAACAACCAGTAT
ATGTAAAA

[a]*Potorous platyops** — COX2 (688 nucleotides):
ATGCCTTATCCAATACAACTGGGCTTTCAAGATGCCACATCCCCTATTATAGAAGAACTAACATATTTCCACGACCATACGCTAATAATTGTTTTCCTAATTA
GCTCCTTAGTATTATATGTCATTATCCTAATACTTACCACAAAACTCACCCATACAAGCACTATAGACGCCCAAGAAGTAGAAACAATTTGAACAATCCTAC
CAGCCGTAATTCTAGTACTAATTGCCCTACCATCCTTACGAATTCTTTACATAATGGACGAAATTTATAACCCCTACCTTACAGTCAAAGCTATAGGTCATCA
ATGATACTGAAGCTATGAGTATACCGATTATGAAGATCTTACATTTGACTCATATATAGTACCCACCCAAGACCTCTCCCCAGGACAGTTCCGACTACTAGA
AGTAGATAATCGACTAGTCCTCCCCATAGAACTCCCAATCCGAATATTAATTTCATCCGAAGATGTTCTCCATGCATGAACTGTCCCATCTCTTGGATTAAAA

GCAGACGCAATTCCAGGACGACTAAATCAAGCCACATTAACATCAACTCGCCCAGGGGTATATTATGGTCAATGCTCAGAAATTTGTGGGTCCAATCACAGT
TTTATACCTATTGTGCTTGAAATAACCACATTGAAATATTTTGAGAAATGATCTTCTATAATGCAATCATTTT

[a]*Potorous platyops** — 16S rRNA (1489 nucleotides):
GATAAATAGGCGCATTAACGTTAGTACCGCAAGGGAAATCTGAAAGACAAGATTTAAAGCACAACAAAGCAAAGATTAACCCTTCTACCTTTTGCATAATG
GTTTAGCTAGTCAAGCCGGACAAAAAGAATTACGCCCGTCTCCCCGAAATCAAGTGAGCTACTATAAAACAGTAAATCAGAACCAACCCGTCTATGTAGCA
AAATAGTGGGAAGATTATATAGTAGAGGTGAAAAGCCTACCGAACTTGAAGATAGCTGGTTGTCCAAAATACGAATTTAAGTTCAACTTTAAATTTAACTAC
AGTACCCACAAACATAATTTAAATTTAAAAGCTAATCAAAAGAGGGACAGCCCTTTTGATTACGTAAACAAACTTTCTAAGAGGATAATGAACCACTCCAAT
CCATTGTAGGCCTAAAAGCAGCCATCAATTAAGAAAGCGTTAAAGCTCAAATTATTTTAATCTTTAATACCTTTAATCAAGCACAACCCCTAAAATAAtCTAT
TGGATGATTCTATaATTtTATAGAAGACATAATGCTAATATCAGTAACATGAAAtTATTTCTCCTaGCACAAGCTTAAATTAGTAACGGAACATCCACTAATAA
TTAACAAATcAATAATcATATCCACACaActAgcayaATTATTATATcaATTGTTAATCCAACACAGGaGTGCATTATTAGGAAAGATTAAAAAGAATAAAAGGAA
CTCGGCAAACACAAACCCCGCCTGTTTACCAAAAACATCACCTCTAGCATAATAAATATTAGAGGCAACGCCTGCCCaGTGAGTTAACacTTTAACGGCCGCG
GTATCCTGACCGTGCAAAGGTAGCATAATCACTTGTCTCCTAAtTAGAGACTTGTATGAATGGCATcACGAGGGTTTAAC??????????????????????????????????????
????????????????????TACAAGACGAGAAGACCCTGTGGAGCTTAAGATTAATAACTTAACCcAACTAAaTATTACCC??????tagg?AACAAAACAA???????CTagaT
TATAATCTTTGGTTGGGGTGACCTCGGAGTACAACACAACCTCCGAATGATTCAArCCmAGATCTACAAATCTAAGTATAATAATATCAGTAATTGACCCATA
TATTGATCAACGGAACAAGTTACCCCAGGGATAACAGCGCAATCCTATTCAAGAGCCCATATCGACAATTAGGGTTTACGACCTCGATGTTGGATCAGGACA
TCCAAATGGTGCACCCGCTATTAATGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACCGGAGAAATCCAGGTCGGTTTCTATCTGTAT
ATTCATTCCTCCCAGTACGAAAGGACAAGAGAAATAAGGCCtACATTACatAGTgAGCCTTaGAAAtAAGATATGAACTTATCTtAATATCCTAACTCAATACTTa
ATACTCTCTAGAACAGAGCT

Supplementary Table S2. *GenBank* accession numbers for nuclear (nDNA) gene sequences used in the phylogenetic analyses. Taxon abbreviations explained in Table S1.

Taxon	ApoB	BRCA1	IRBP	RAG1	vWF
			Phalangeridae (outgroup)		
Pot	AF548431	AY243449	AY243436	AY243393	AY243410
			Burramyidae (outgroup)		
Ccn	GU566712	GU566715	GU566718	AY125036	GU566724
			Hypsiprymnodontidae		
Hms	FJ603118	FJ603120	FJ603129	JN414879	FJ603139
			Potoroidae		
Arf	EU160444	EU160439	EU160448	EU160451	EU160454
Ccp*	–	–	–	–	–
Bah*	–	–	–	–	–
Bgm	FJ603114	FJ603168	FJ603196	FJ603223	FJ603250
Bls	–	–	–	–	–
Bpn	FJ603145	FJ603169	FJ603197	FJ603224	FJ603251
Btp	JX104618			JX104593	JX104618
Pgb	JX104616	JX104603		JX104591	
Plg	FJ603163	FJ603191	FJ603217	FJ603245	FJ603272
Ppl*	–	–	–	–	–
Pta	FJ603164	FJ603192	FJ603218	FJ603246	FJ603273
Ptd	JX104615	JX104594	–	JX104580	–
Pts	JX104612	JX104599	–	JX104587	–
			Macropodidae		
Ddo	FJ603146	FJ603170	FJ603198	FJ603222	FJ603252
Dlm	MK211389	MH197811	MK211391	MK197843	MK211392
Dhg	–	–	–	–	–
Dvh	FJ603147	FJ603173	FJ603200	FJ603227	FJ603255
Lcs	FJ603148	FJ603174	FJ603201	FJ603228	FJ603256
Lhs	FJ603142	FJ603175	FJ603202	FJ603229	FJ603257
Lfs	FJ603149	FJ603176	FJ603203	FJ603230	FJ603258
Mfg	FJ603143	FJ603180	FJ603207	FJ603234	FJ603262
Mgg	FJ603153	FJ603181	AJ429135	FJ603235	AJ224670
Neg	FJ603152	FJ603179	FJ603206	AY059703	FJ603261
Npm	FJ603155	FJ603183	FJ603209	FJ603237	FJ603264
Obn	–	–	–	–	–
Orb	FJ603157	FJ603185	FJ603211	FJ603239	FJ603266

Taxon (Continued)	ApoB	BRCA1	IRBP	RAG1	vWF
Orf	FJ603117	FJ603121	FJ603127	FJ607154	FJ603138
Pby	–	JQ042190	–	–	–
Pxt	FJ603162	FJ603190	FJ603216	FJ603244	FJ603271
Pak*	–	–	–	–	–
Sbr	FJ603165	FJ603193	FJ603219	FJ603247	FJ603274
Soc*	–	–	–	–	–
Tbl	FJ603166	–	–	–	–
Wbc	FJ603167	–	FJ603221	–	FJ603276
Ddo	FJ603146	FJ603170	FJ603198	FJ603222	FJ603252

Supplementary Table S3. Gene partition models used in the phylogenetic analyses.

Partition	Model	P invar	Gamma
mitogenome	GTR + I + Γ	0.530	0.9700
ApoB	GTR + Γ	–	0.7370
BRCA1	GTR + Γ	–	0.7880
IRBP	GTR + Γ	–	0.3030
RAG 1	GTR + Γ	–	0.2390
vWF	GTR + I + Γ	0.3930	0.8250

Supplementary Table S4. *PAUP 4.0b10[51] constraint tests for monophyly of *Caloprymnus campestris** (Ccp*) with either *Aepyprymnus rufescens* (Arf)[49,50], or the species of *Potorous* (Pts)[27,46].** Abbreviations: Con, constraint; Dif, difference; KH, Kishino-Hasegawa test; lnL, $\log_n$ likelihood; SH, Shimodaira-Hasegawa test; SH-AU, Shimodaira Approximately Unbiased test; w-SH, weighted Shimodaira-Hasegawa test. *Extinct.

Con	-lnL	Dif	KH-sd	KH-T	KH-P	SH	w-SH	SH-AU
Ccp*+Arf	128651.60066	131.89083	33.562	3.930	0.0001***	0.0001**	0.0001**	0.0001***
Ccp*+Pts	128971.98974	452.27991	49.539	9.130	<0.0001***	<0.000**	<0.000**	<0.000**

Supplementary Table S5. Estimated divergence times (Ma) with confidence intervals for crown macropodoid clades. Results were derived from supplementary analyses using the ^{P}partitioned mitogenome/mtDNA/nDNA with ^{C}*Wallabia bicolor* constrained[12,42,80,81], the $^{N-P}$non-partitioned mitogenome/mtDNA/nDNA, ^{P}partitioned versus $^{N-P}$non-partitioned mitogenome, and the nDNA dataset run separately. See figure 2 and table 2 for an explanation of the node numbers: 4† = *Potorous longipes* v. *Potorous tridactylus*; 6† = *Potorous gilberti* v. *P. longipes* + *P. tridactylus*; 6†† = *P. longipes* v. *P. tridactylus*; 12† = *Bettongia lesueur* v. *Bettongia penicillata*; 15† = *Simosthenurus occidentalis** v. other Macropodidae; 16† = *S. occidentalis** v. *Lagostrophus fasciatus*; 17† = Dorcopsini v. Macropodini; 24† = *Onychogalea unguifera* v. other Macropodinae; 30† = *Wallabia bicolor* v. *Osphranter* spp. + *Notamacropus* spp.; 30†† = *Osphranter robustus* + *Osphranter bernardus* v. other Macropodini; 30††† = *O. robustus* v. other Macropodini; 30†††† = *O. bernardus* v. other Macropodini; 32† = *Osphranter rufus* v. *Notamacropus* spp. *Extinct.

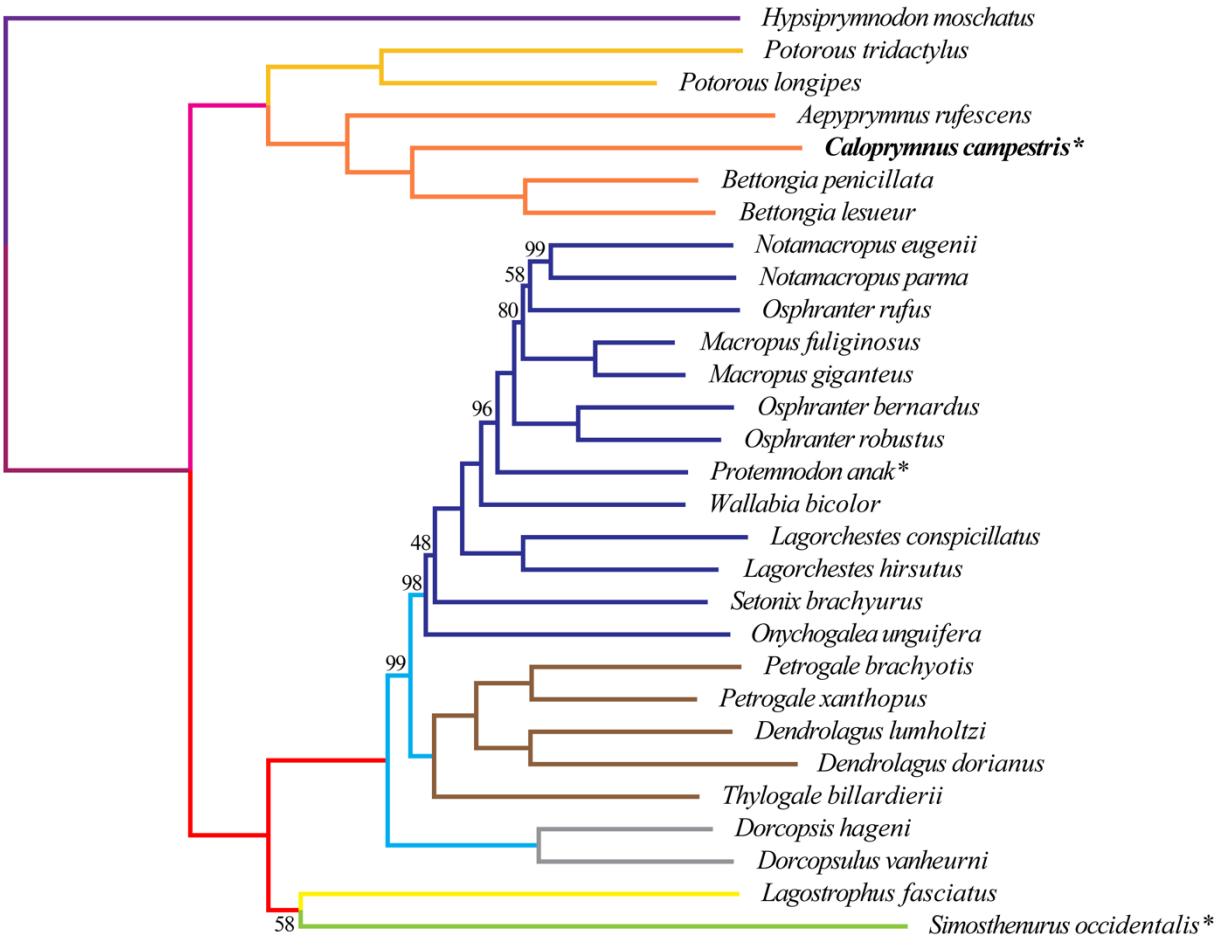
Node	^{PC}Mitogenome/ mtDNA/nDNA	$^{N-P}$Mitogenome/ mtDNA/nDNA	^{P}Mitogenome	$^{N-P}$Mitogenome	nDNA
1	30.1 (24.29-36.01)	30.63 (24.83-36.98)	31.35 (24.94-37.67)	31.77 (24.71-38.52)	37.98 (29.6-46.42)
2	21.8 (17.88-25.72)	21.79 (17.58-26)	21.92 (17.92-25.79)	22.07 (17.91-26.28)	22.9 (16.56-27.33)
3	18.49 (14.72-22.49)	18.79 (15.23-23.05)	18.08 (14.68-21.6)	17.78 (13.72-22.36)	19.03 (15.06-23.05)
4	13.04 (10.43-15.71)	13.35 (9.59-16.76)	–	–	–
4†	–	–	12.48 (9.61-15.43)	12.43 (9.05-16.22)	–
5	11.37 (8.84-14.1)	11.66 (8.64-14.82)	–	–	–
6	9.61 (7.36-12.08)	9.87 (3.97-12.37)	–	–	–
6†	–	–	–	–	11.9 (8.84-14.8)
6††	–	–	–	–	8.54 (6.08-10.95)
7	7.38 (5.25-9.65)	7.56 (4.89-10.42)	–	–	3.86 (2.1-5.8)
8	2.14 (1.41-3.12)	2.11 (0.98-3.25)	–	–	1.32 (0.3-2.5)
9	13.99 (9.86-18.67)	14.59 (11.28-18.25)	14.52 (11.04-18.43)	14.48 (10.05-19.19)	10.65 (7.33-13.92)
10	11.65 (7.84-16.91)	12.14 (9.12-15.78)	12.16 (9.72-14.76)	11.87 (6.92-15.66)	–
11	8.44 (4.28-13.06)	8.51 (5.59-12.25)	–	–	–
12	7.37 (5.35-9.95)	7.37 (1.33-9.35)	–	–	–
12†	–	–	6.98 (4.65-9.5)	6.96 (4.16-9.84)	–
13	2.39 (1.54-3.42)	2.4 (1.3-5.5)	–	–	3.49 (2.03-5.12)
14	1.69 (0.53-2.43)	1.67 (0.02-2.25)	–	–	1.5 (0.29-2.88)
15	–	18.92 (15.13-23.12)	–	–	–
15†	19.05 (15.49-22.71)	–	18.73 (15.16-22.4)	18.94 (14.85-22.74)	–
16	–	17.29 (13.26-21.16)	–	–	19.86 (15.59-24.11)
16†	17.59 (14.12-21.03)	–	16.83 (8.12-21.47)	17.61 (14-21.11)	–
17	13.44 (10.8-16.07)	13.31 (10.28-20.94)	13.41 (10.38-16.46)	13.04 (10.34-15.76)	–
17†	–	–	–	–	10.36 (8.02-12.94)
18	7.19 (5.17-9.19)	6.83 (4.25-9.2)	6.66 (3.16-9.02)	6.58 (0.62-8.66)	3.36 (1.82-5.02)
19	12.47 (10.11-14.86)	12.26 (10.21-14.38)	12.22 (9.65-14.75)	12.08 (9.91-14.19)	11.01 (8.48-13.64)
20	11.23 (8.95-13.6)	11.09 (5.82-13.4)	11.22 (9.02-13.44)	11.01 (8.75-13.59)	8.82 (6.58-11.12)
21	9.58 (7.42-11.82)	9.45 (5.27-11.38)	9.42 (7.58-11.23)	9.29 (7.15-11.79)	7.4 (5.49-9.35)
22	7.28 (5.38-9.33)	7.17 (2.31-9.2)	7.04 (5.11-8.92)	6.8 (1.04-9.12)	4.66 (2.83-6.62)

Node (Continued)	^{PC}Mitogenome/ mtDNA/nDNA	$^{N-P}$Mitogenome/ mtDNA/nDNA	^{P}Mitogenome	$^{N-P}$Mitogenome	nDNA
23	7.56 (5.7-9.42)	7.43 (3.81-9.25)	7.46 (5.8-9.06)	7.04 (0.05-8.71)	5.05 (3.18-7.08)
24	11.82 (9.55-14)	11.68 (9.48-13.99)	11.29 (9-13.66)	11.32 (9.29-13.34)	–
24†	–	–	–	–	11.9 (9.14-14.88)
25	11.25 (9.29-13.27)	11.15 (9.27-13.08)	10.92 (8.93-12.89)	10.73 (8.18-13.43)	9.74 (7.38-12.2)
26	10.31 (8.31-12.26)	10.11 (8.34-11.99)	9.82 (8.02-11.63)	9.62 (7.32-12.36)	9.04 (6.82-11.38)
27	7.27 (5.11-9.75)	7.29 (5.27-9.39)	7.06 (3.2-9.49)	7.46 (5.9-9.07)	4.73 (3.06-6.52)
28	–	9.27 (7.63-10.98)	8.98 (7.34-10.7)	9.02 (7.37-10.67)	–
29	8.96 (7.14-10.71)	8.65 (6.98-10.59)	8.04 (5.09-10.37)	8 (5.28-10.63)	–
30	8.44 (6.68-10.05)	7.94 (6.33-9.78)	6.94 (3.31-8.83)	6.97 (5.51-8.4)	8.18 (6.05-10.42)
30†	8.32 (6.6-9.96)	–	–	–	7.39 (5.41-9.47)
30††	–	–	7.33 (4.09-9.44)	–	–
30†††	–	–	–	7.51 (6.09-9.05)	–
30††††	–	–	–	7.24 (4.62-9.56)	–
31	3.83 (2.72-4.98)	3.63 (1.5-5.04)	3.37 (1.16-4.7)	3.28 (0.08-4.29)	1.24 (0.46-2.12)
32	7.82 (6.34-9.39)	7.62 (6.03-9.39)	–	–	6.72 (4.88-8.7)
32†	–	–	6.55 (2.63-8.61)	6.43 (2.92-8.49)	
33	6.57 (5.13-8)	6.43 (5-7.91)	5.68 (0.72-7.26)	5.74 (3.25-7.75)	4.28 (2.75-5.91)
34	7.26 (5.87-8.74)	7.05 (1.87-8.97)	–	–	2.98 (1.57-4.47)
35	5.23 (4.1-6.4)	5.02 (0.93-6.28)	4.91 (2.1-6.43)	–	–

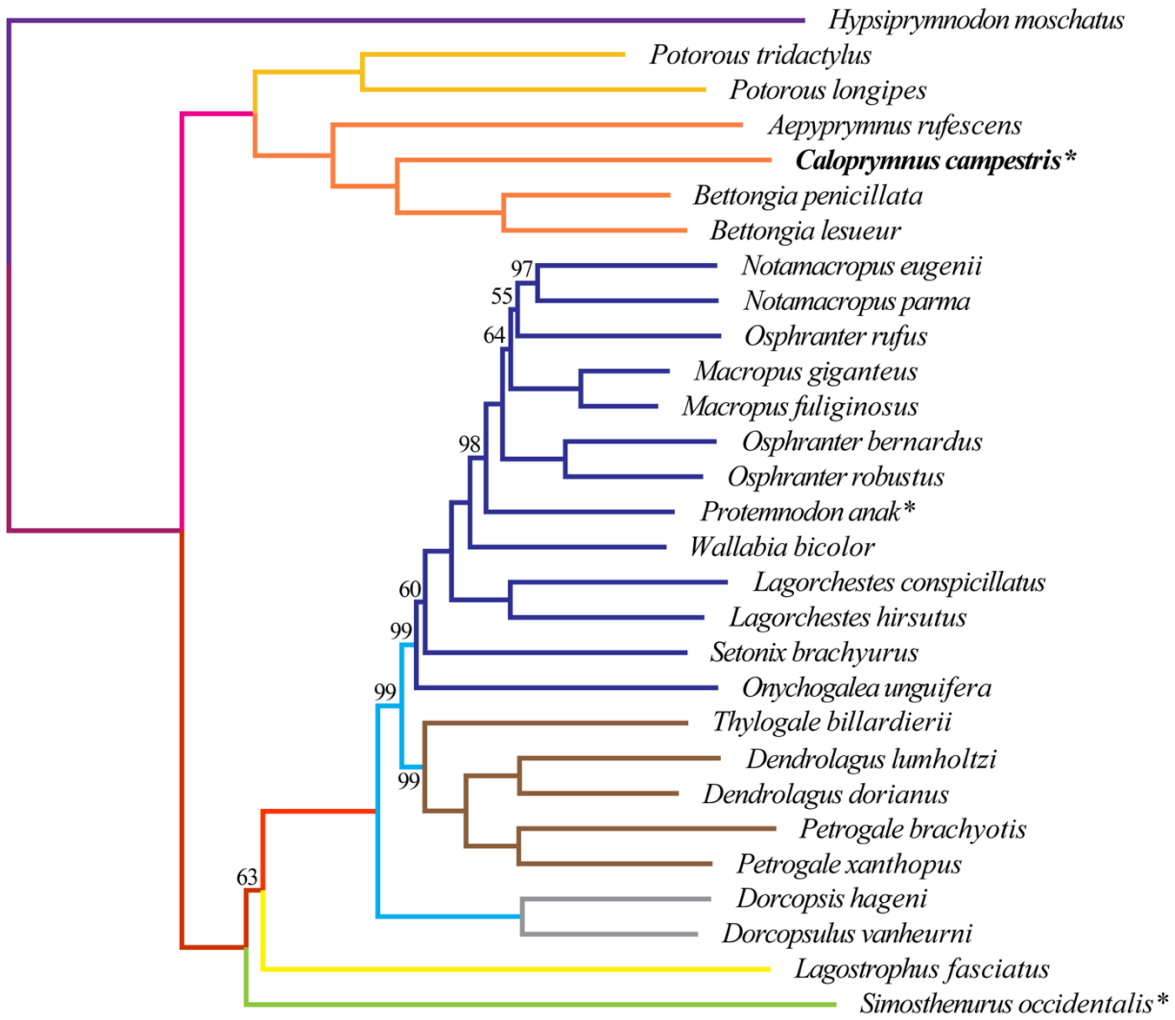
Supplementary Table S6. Comparative Cyt *b* K2P values for potoroid species and subspecies. Taxon abbreviations are explained in Table S1.

	Pta	**Ptd**	**Pts**	**Pgb**	**Ppl***	**Plg**	**Bpn**	**Btp**	**Bgm**	**Bls**	**Ccp***	**Arf**
Pta	–	1.928	4.211	5.051	6.540	6.083	7.601	6.902	7.602	8.062	8.575	7.556
Ptd	–	–	3.540	2.693	4.089	5.843	7.713	7.028	7.603	7.932	8.432	7.990
Pts	–	–	–	4.998	4.998	5.685	7.270	6.736	7.266	8.128	8.248	7.298
Pgb	–	–	–	–	5.730	3.589	8.823	8.023	8.244	9.301	9.424	8.729
Ppl*	–	–	–	–	–	6.540	7.463	6.663	7.358	8.288	8.431	7.869
Plg	–	–	–	–	–	–	7.728	8.004	7.740	7.353	7.961	7.291
Bpn	–	–	–	–	–	–	–	2.485	1.712	6.885	6.906	7.231
Btp	–	–	–	–	–	–	–	–	2.040	4.252	7.147	7.952
Bgm	–	–	–	–	–	–	–	–	–	4.252	7.147	7.953
Bls	–	–	–	–	–	–	–	–	–	–	6.995	8.434
Ccp*	–	–	–	–	–	–	–	–	–	–	–	8.093
Arf	–	–	–	–	–	–	–	–	–	–	–	–

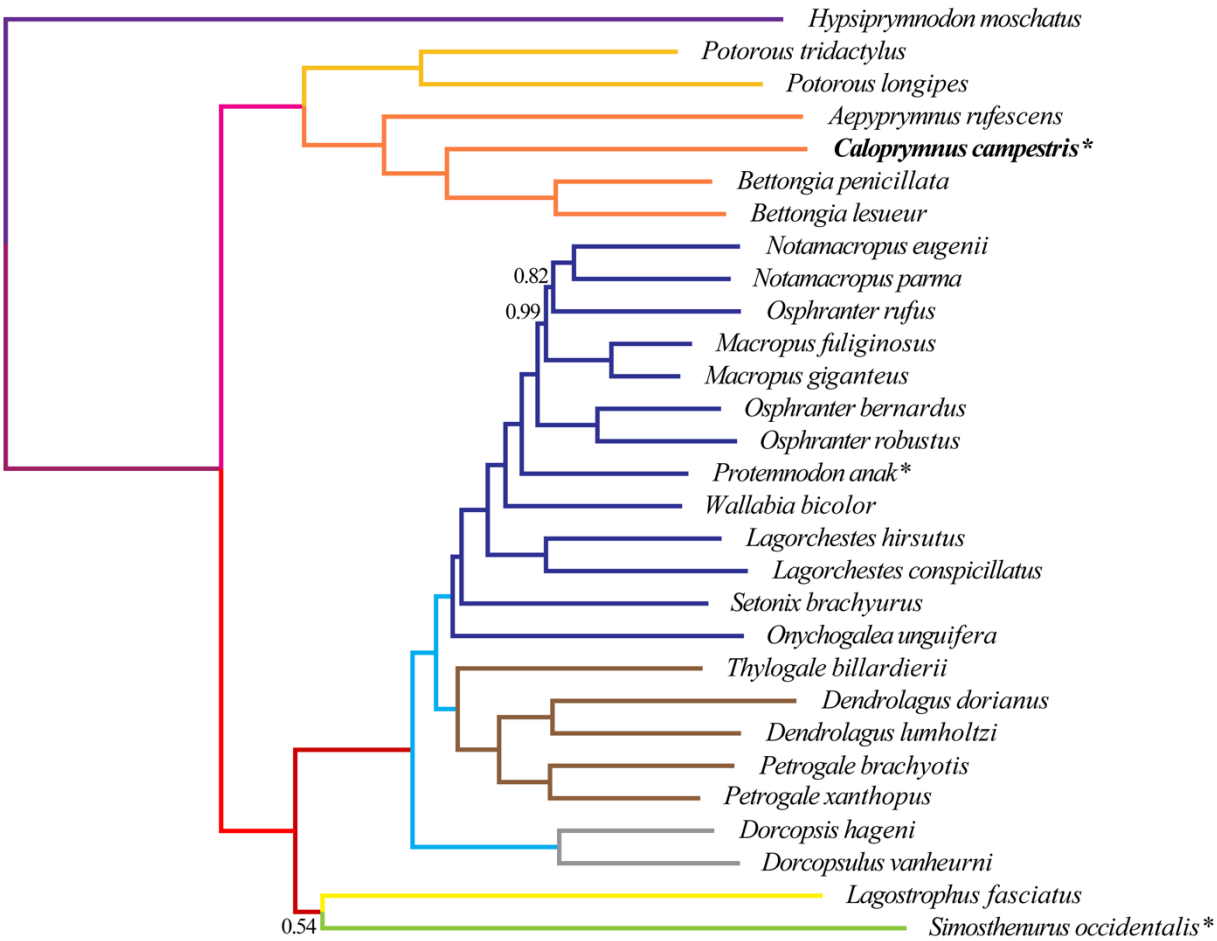
Supplementary figures



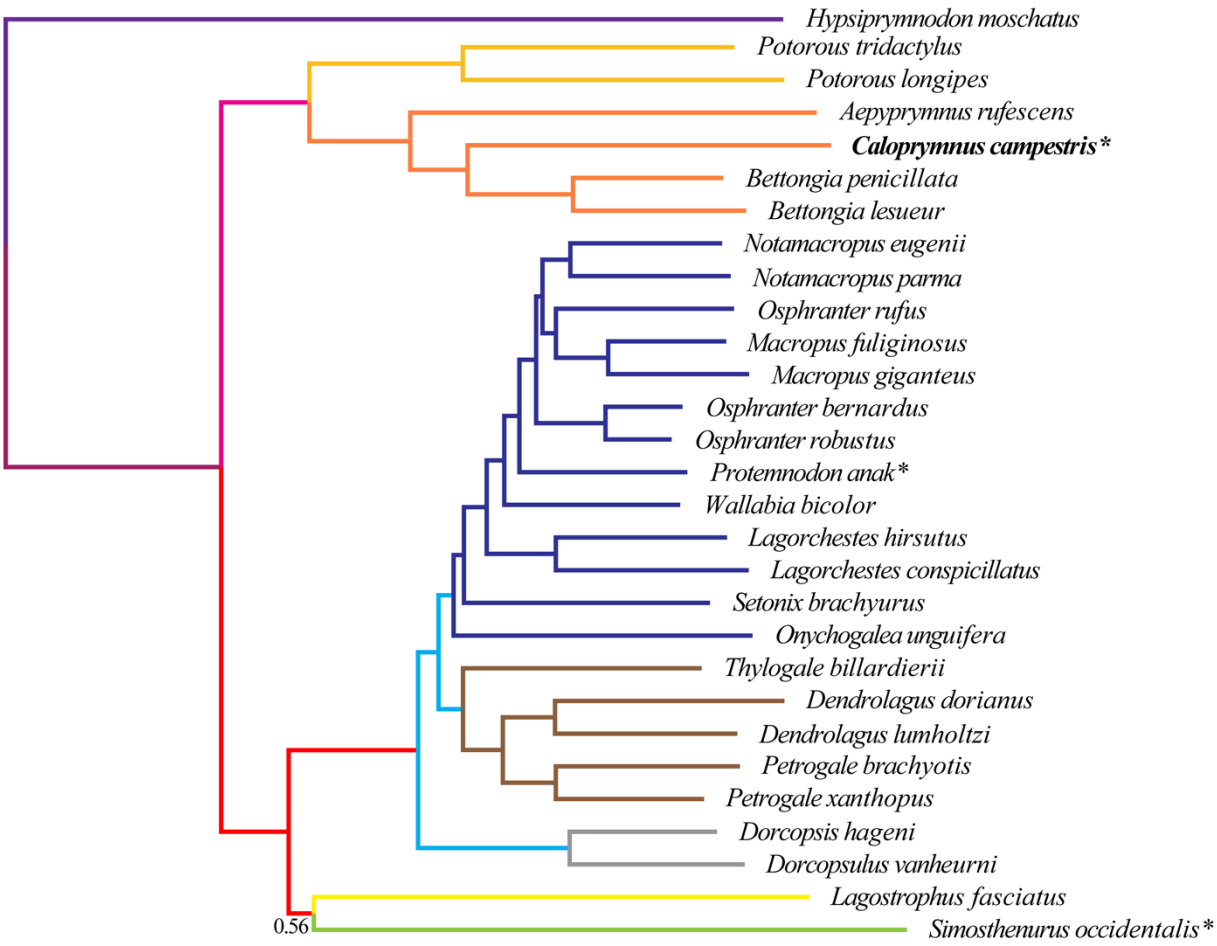
Supplementary Figure S1. Maximum likelihood consensus tree of the partitioned mitogenome dataset generated by *RAxML* 7.2.8[34]. Bootstrap support values (<100%) are indicated at relevant nodes. Branch colours denote major clades: Hypsiprymnodontidae (purple); Macropodia, new clade (burgundy); Potoroidae (pink); Potoroinae (orange); Bettonginae (ochre); Macropodidae (red); Sthenurinae (green); Lagostrophinae (yellow); Macropodinae (light blue); Dorcopsini (grey) Dendrolagini (brown); Macropodini (dark blue). *Extinct taxa.



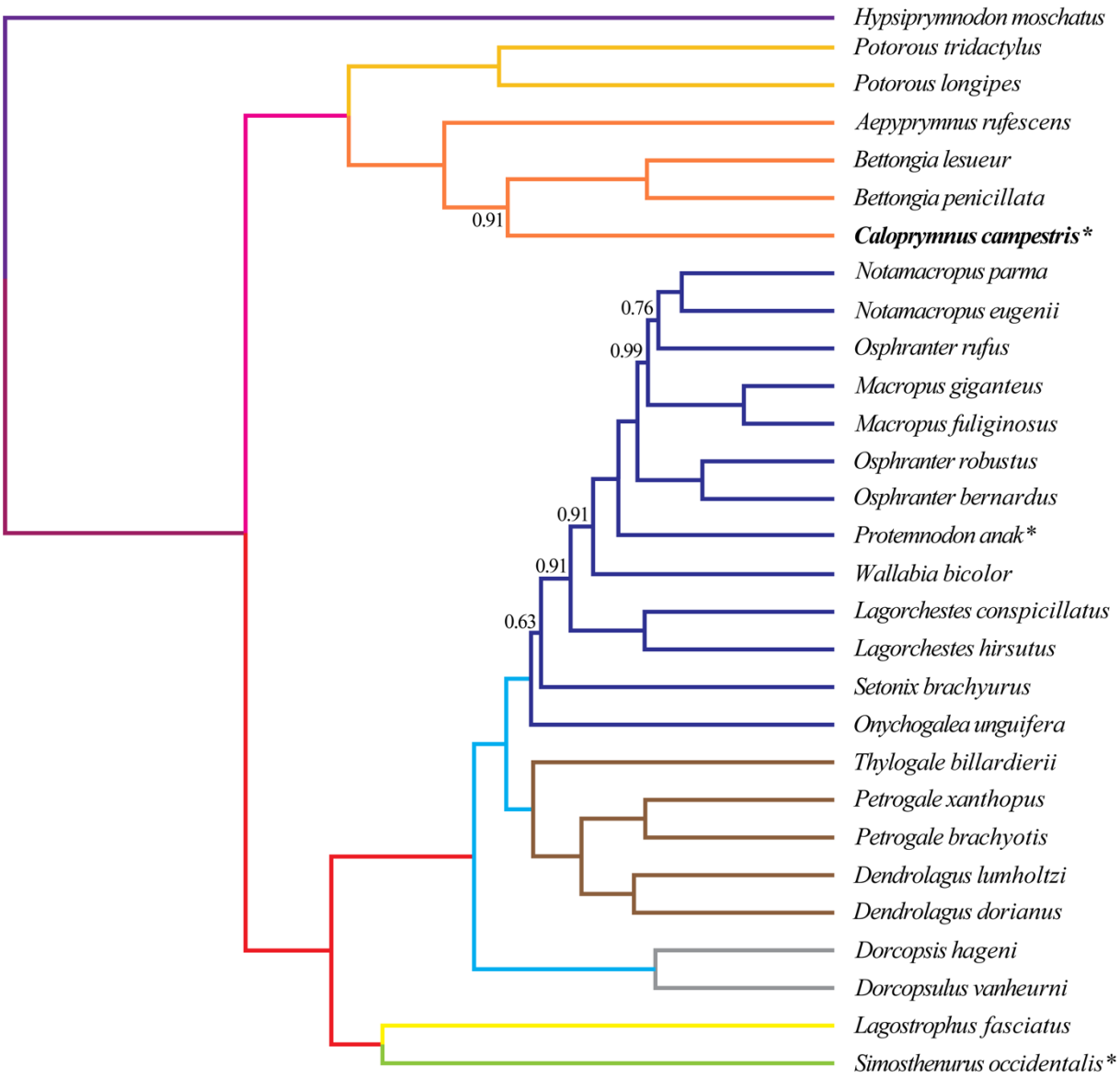
Supplementary Figure S2. Maximum likelihood consensus tree of the non-partitioned mitogenome dataset generated by *RAxML* 7.2.8. Bootstrap support values (<100%) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



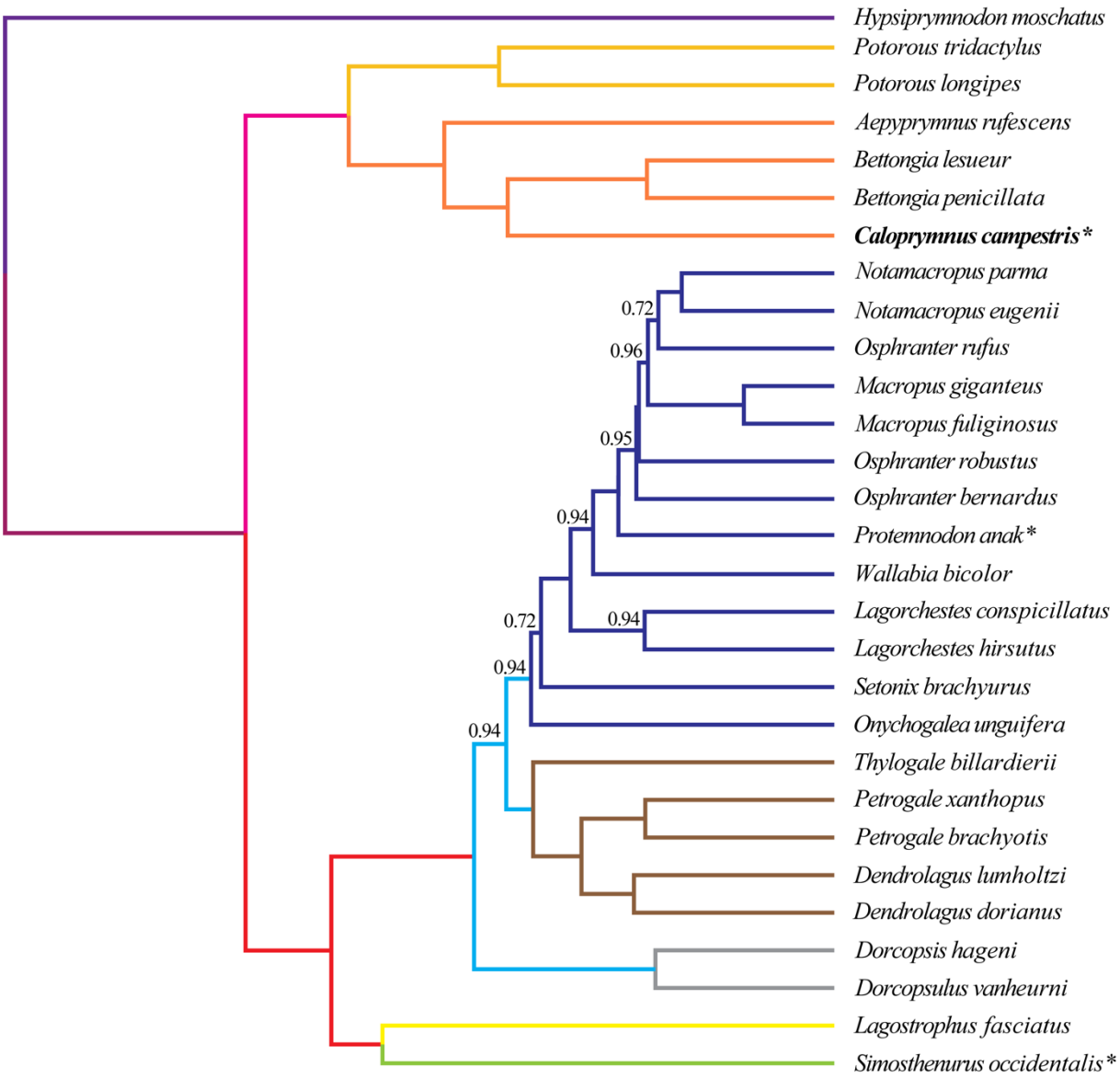
Supplementary Figure S3. Bayesian consensus tree of the partitioned mitogenome dataset generated by *MrBayes* 3.2.7[35]. Bayesian Posterior Probability (BPP) support values (<1.0) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



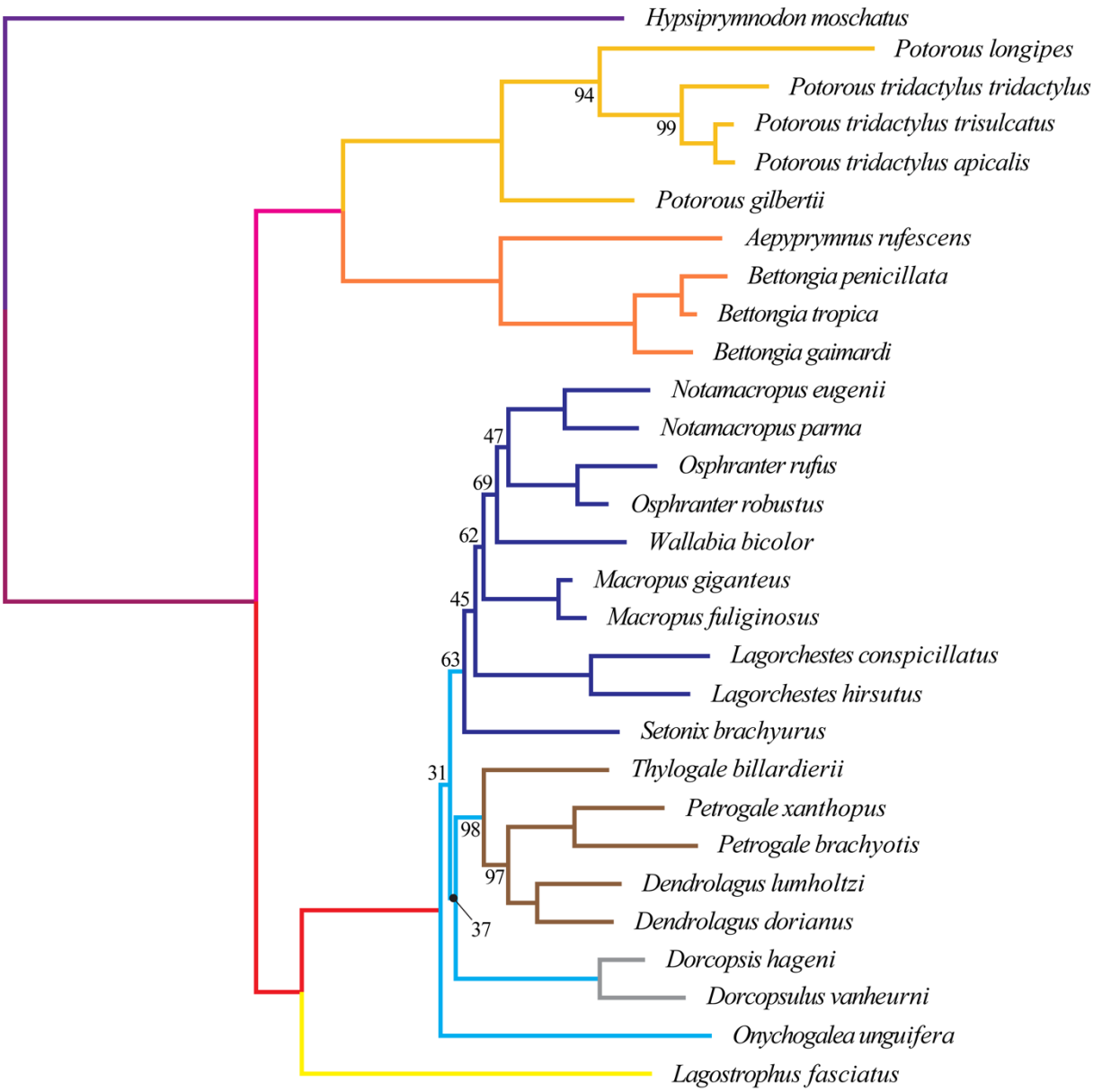
Supplementary Figure S4. Bayesian consensus tree of the non-partitioned mitogenome dataset generated by *MrBayes* 3.2.7. BPP support values (<1.0) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



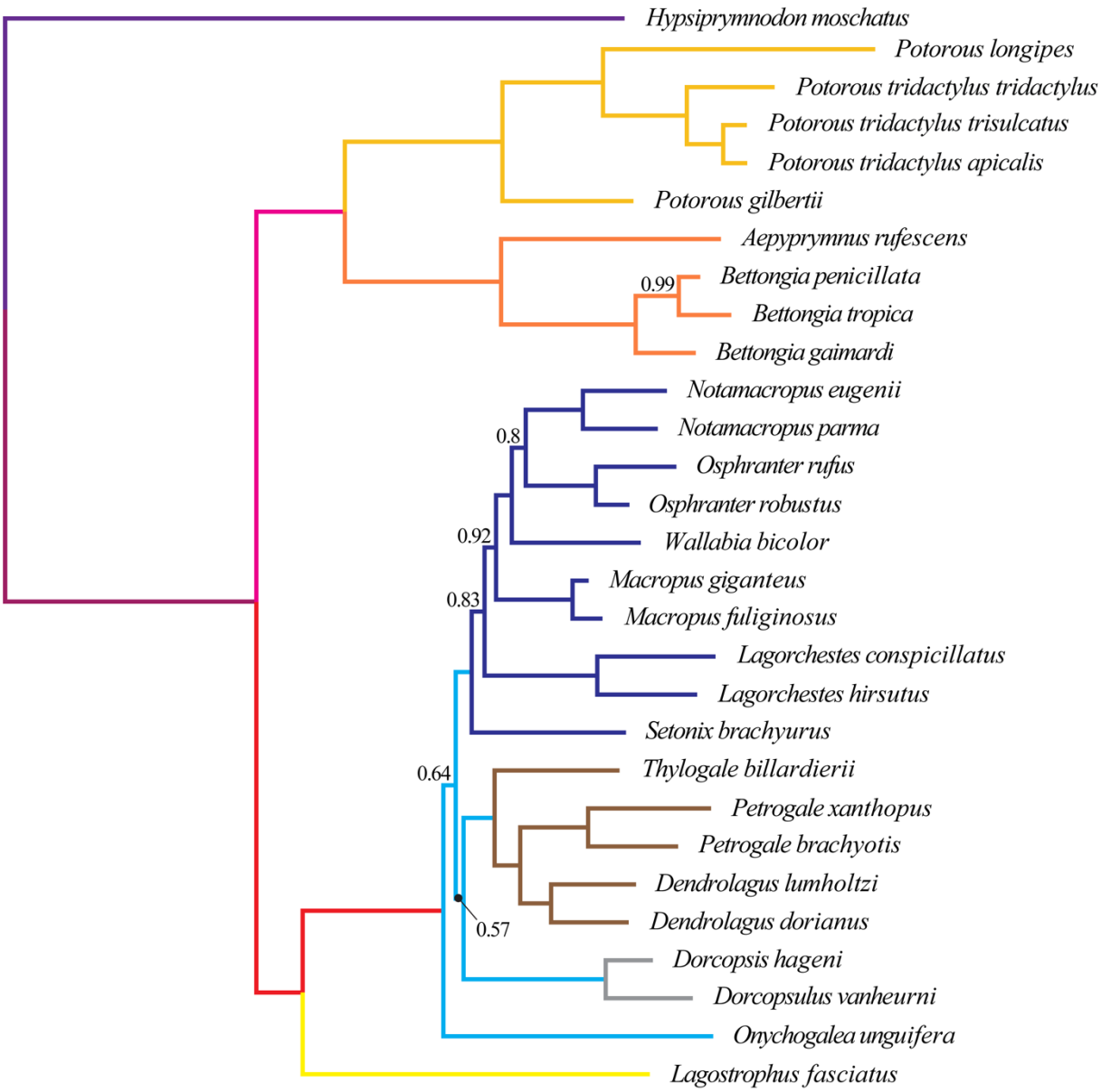
Supplementary Figure S5. Bayesian consensus tree of the partitioned mitogenome dataset generated by *Beast 1.8.2*[36]. BPP support values (<1.0) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



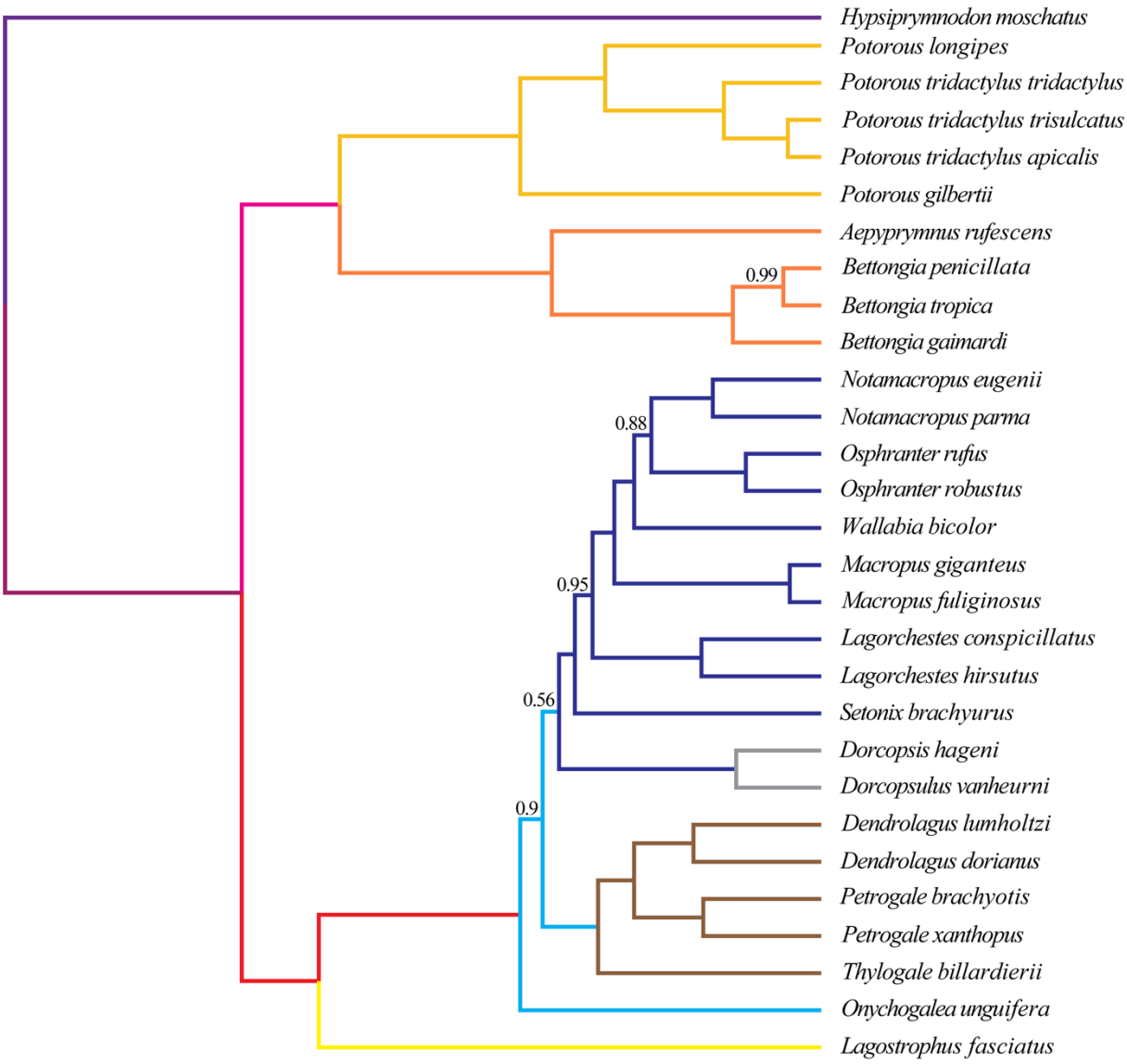
Supplementary Figure S6. Bayesian consensus tree of the non-partitioned mitogenome dataset generated by *Beast 1.8.2*. BPP support values (<1.0) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



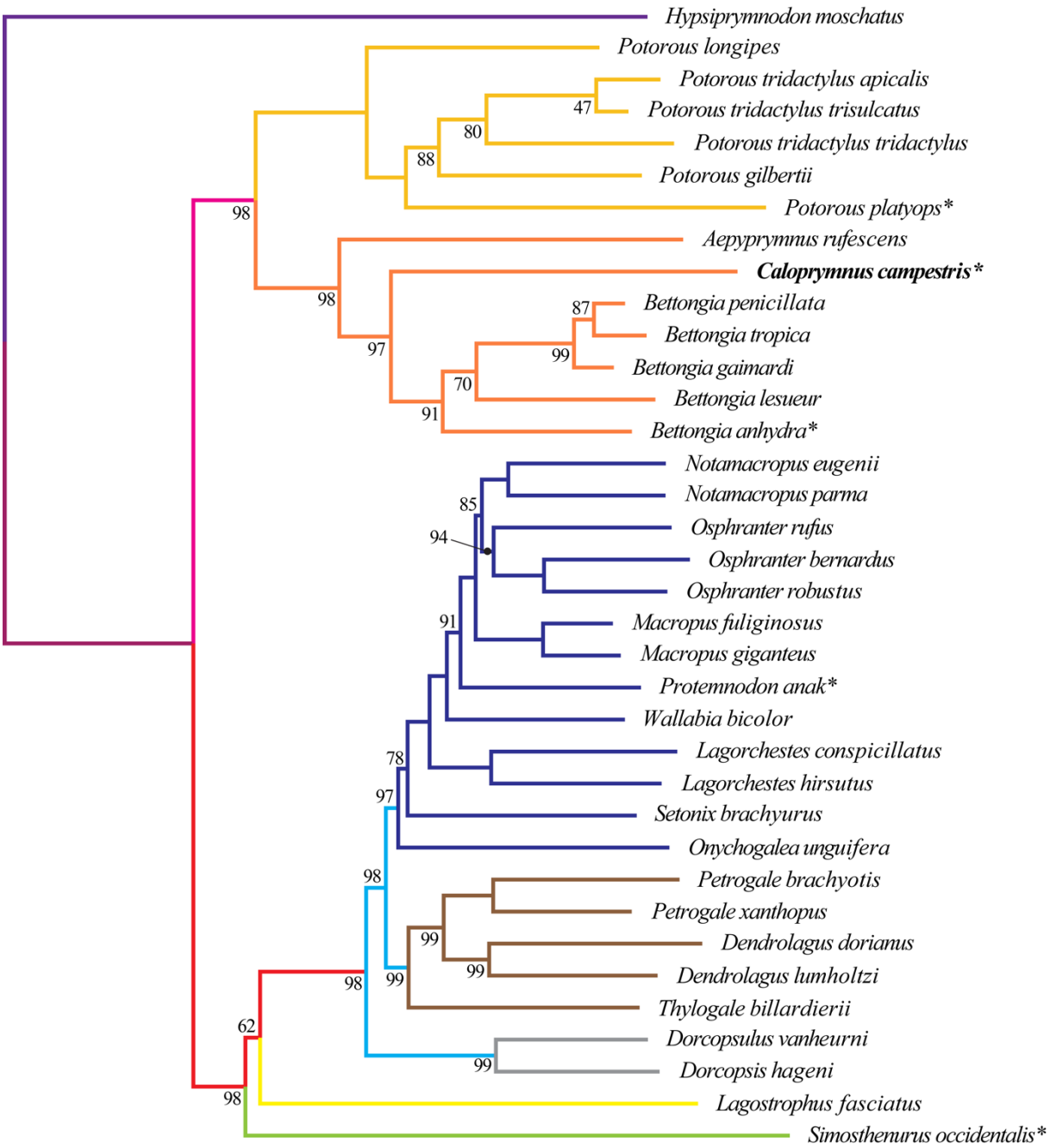
Supplementary Figure S7. Maximum likelihood consensus tree of the nuclear gene sequence (nDNA) dataset generated by *RAxML* 7.2.8. Bootstrap support values (<100%) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



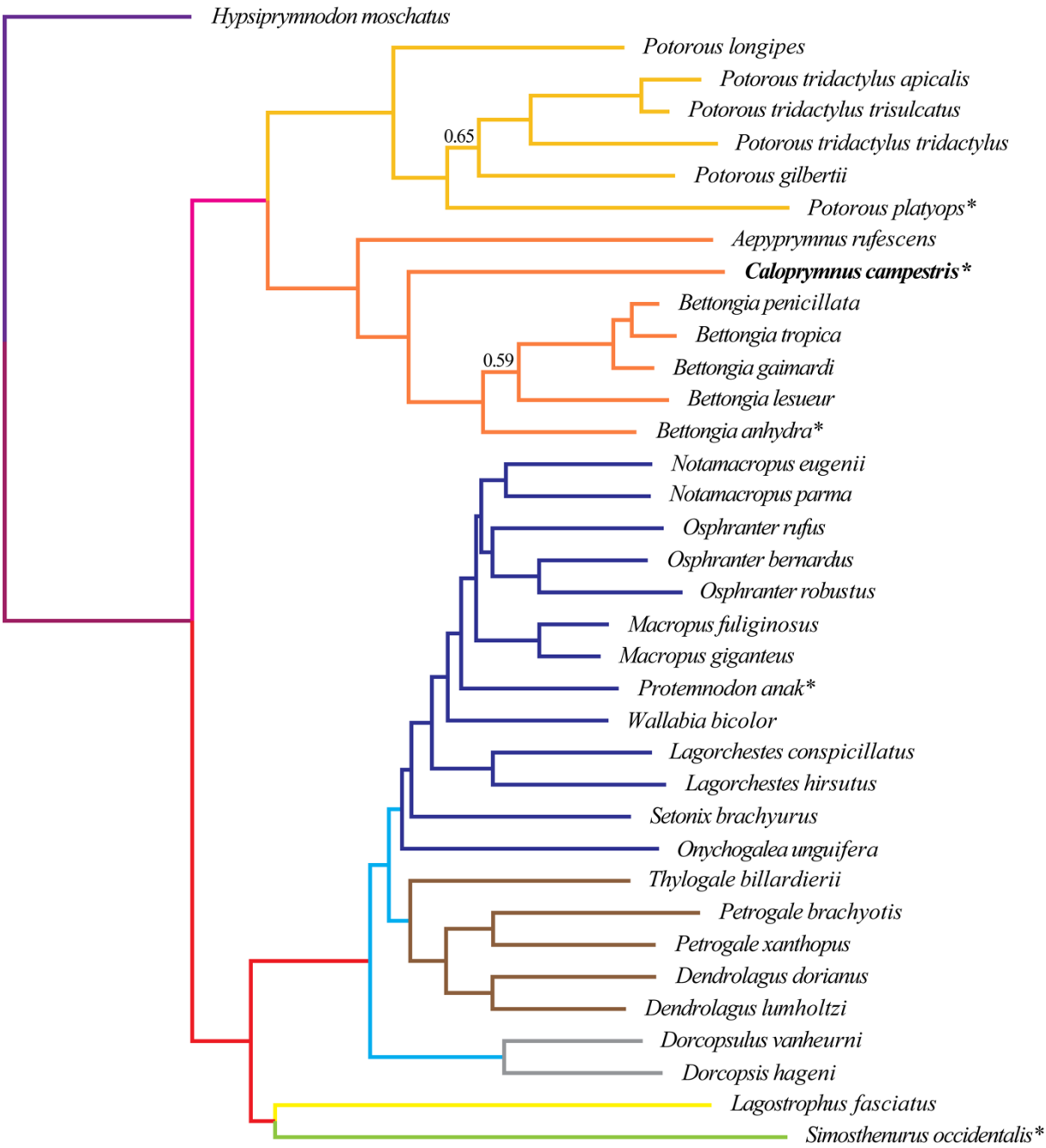
Supplementary Figure S8. Bayesian consensus tree of the nDNA dataset generated by *MrBayes* 3.2.7. BPP support values (<1.0) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



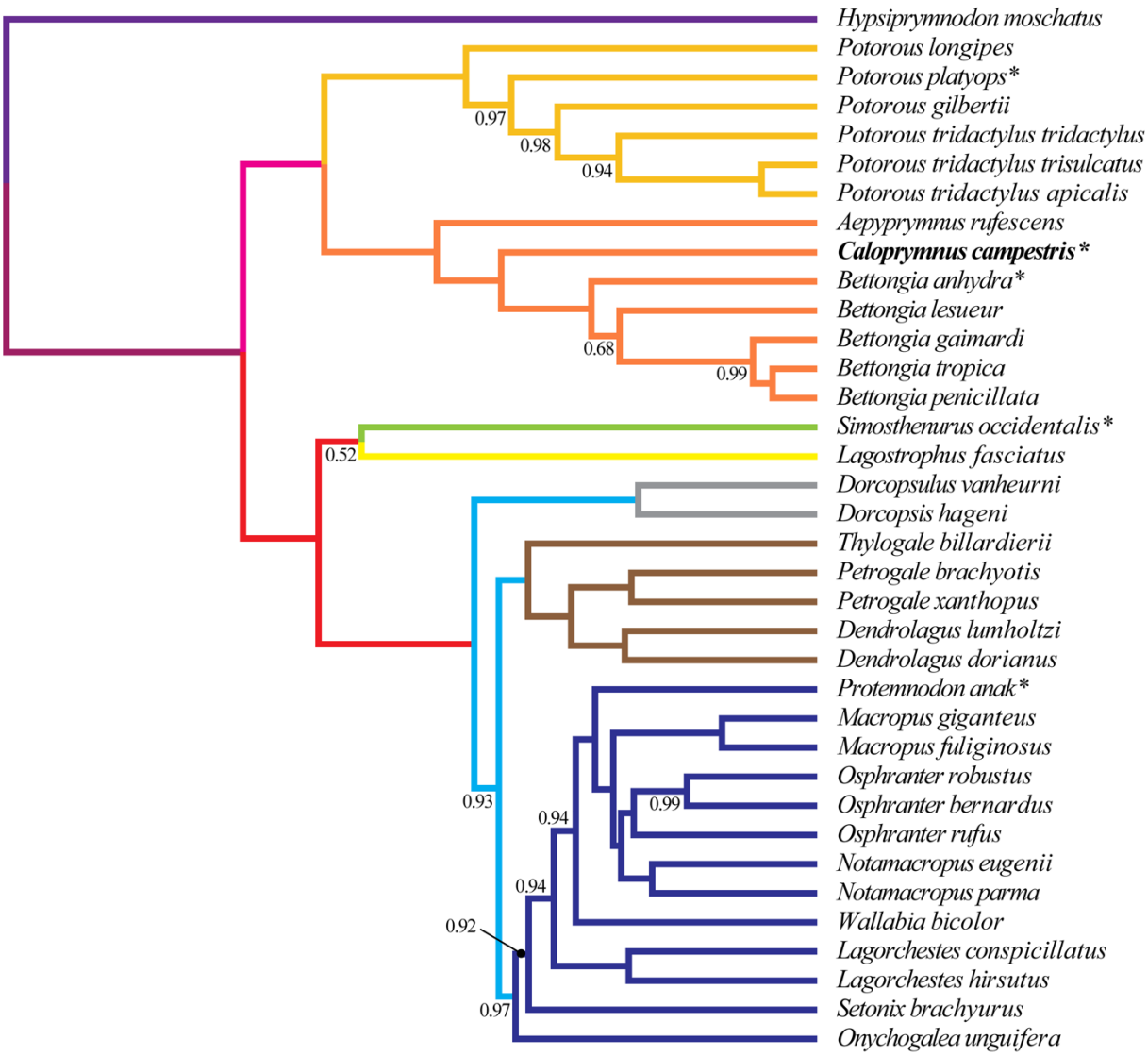
Supplementary Figure S9. Bayesian consensus tree of the nDNA dataset generated by *Beast 1.8.2*. BPP support values (<1.0) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



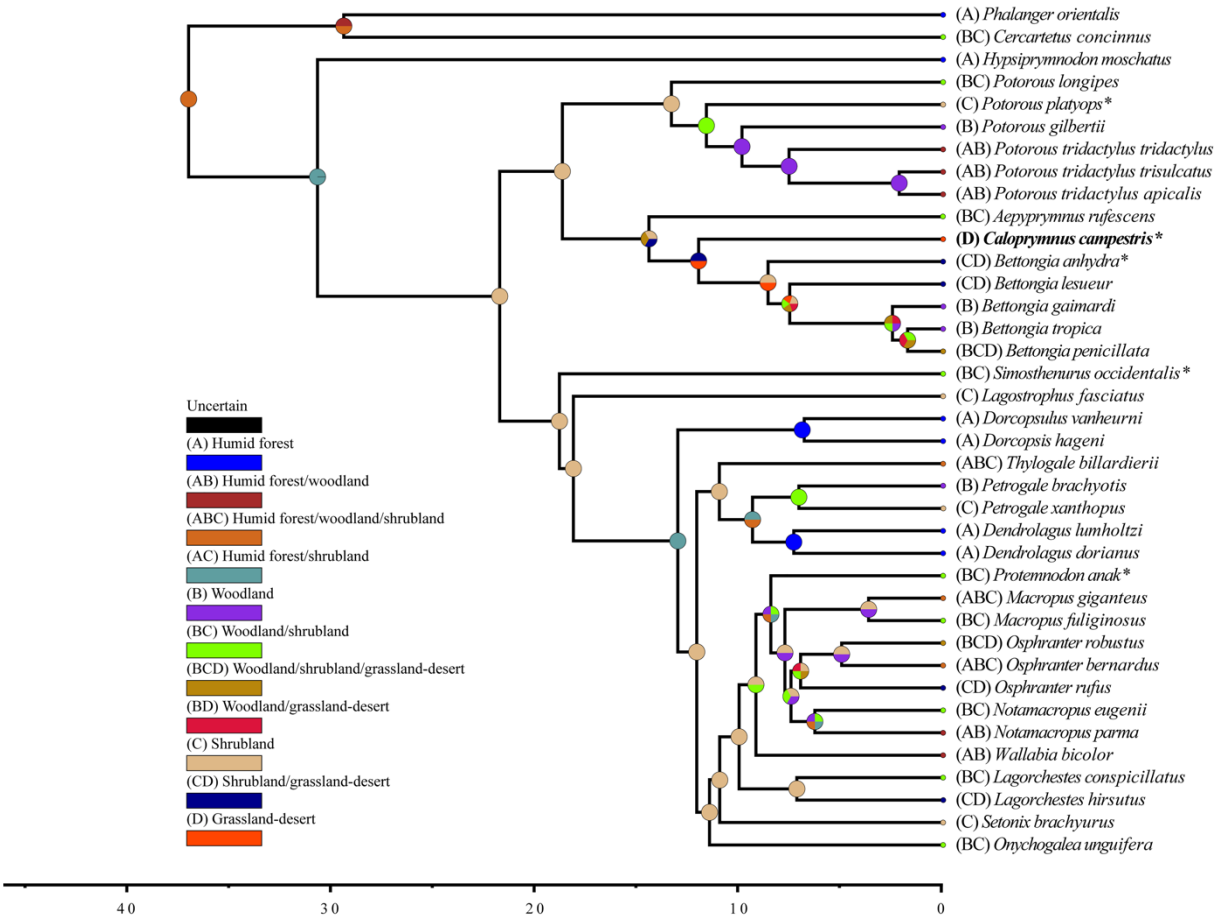
Supplementary Figure S10. Maximum likelihood consensus tree of the non-partitioned mitogenome/mitochondrial gene sequence (mtDNA)/nDNA dataset generated by *RAxML* 7.2.8. Bootstrap support values (<100%) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



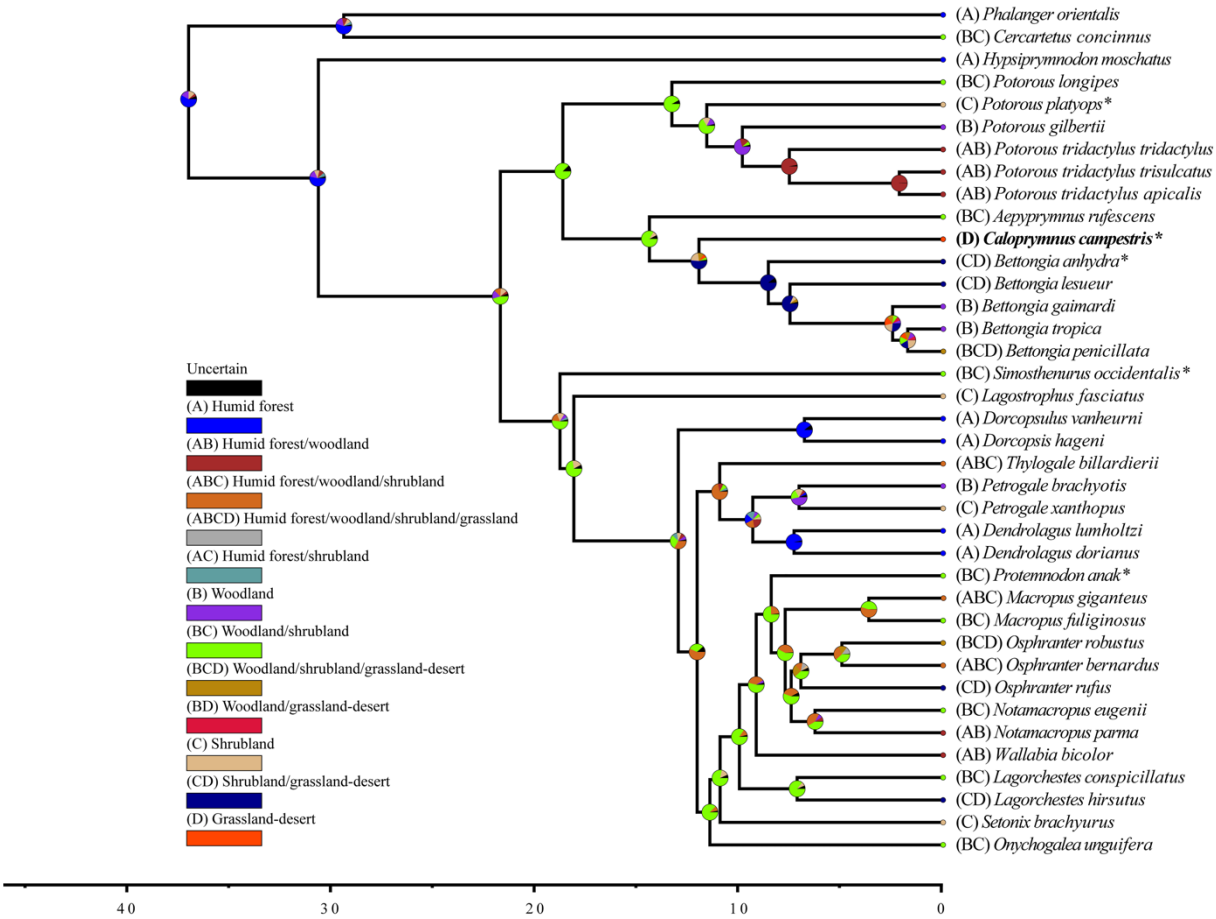
Supplementary Figure S11. Bayesian consensus tree of the non-partitioned mitogenome/mtDNA/nDNA dataset generated by *MrBayes* 3.2.7. BPP support values (<1.0) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



Supplementary Figure S12. Bayesian consensus tree of the non-partitioned mitogenome/mtDNA/nDNA dataset generated by *Beast 1.8.2*. BPP support values (<1.0) are indicated at relevant nodes. Branch colours denoting major clades follow Supplementary Figure S1.



Supplementary Figure S13. Node dated (Ma) consensus tree of the partitioned mitogenome/mtDNA/nDNA dataset generated by *Beast* with S-DIVA ancestral area optimisations from *RASP 4*[38]. Habitat codes are listed for each taxon (right) and explained in the text.



Supplementary Figure S14. Node dated (Ma) consensus tree of the partitioned mitogenome/mtDNA/nDNA dataset generated by *Beast* with Bayesian Binary MCMC (BBM) ancestral area optimisations from *RASP 4*. Habitat codes are listed for each taxon (right) and explained in the text.

Systematics

The higher-level systematics of Macropodiformes (the most inclusive clade containing Macropodoidea[14]) has long been contentious[26,82], with multiple ranking and phylogeny-based classification schemes currently in use[3,14,26,46,82]. To minimise ambiguities in our description of crown macropodoid inter-relationships, we therefore propose new formal phylogenetic definitions for Macropodiformes and all higher-level constituent clades that have received robust analytical support. Our preferred taxonomic nomenclature (Table 1) builds on the schemes of Kear & Cooke[26], Jackson & Groves[72], Den Boer & Kear[14], Eldridge *et al.*[81] and Beck *et al.*[45]. Our clade-type designations follow the conceptual framework of Lee[83] where node-based definitions are implicated by extant taxa only, as opposed to stem-based definitions which employ both extant and fossil taxa.

Unranked clade Macropodia, new clade

Although elsewhere treated as an equivalent to Macropodidae[3,9,60,84] or Macropodoidea[45], our concept of Macropodoidea *sensu stricto* (Table 1) as the clade encapsulating the entire crown radiation to the exclusion of demonstrable stem taxa, such as *Palaeopotorous priscus*[14] and possibly the extinct balbarids ("fanged kangaroos"[82])[26,85–88], necessitates designation of a new name[46] for the most inclusive subclade containing Potoroidae + Macropodidae (kangaroos, wallaroos, wallabies, pademelons, tree-kangaroos, bettongs and potoroos) to the exclusion of Hypsiprymnodontidae (rat-kangaroos)[82]. This clade is unanimously resolved by all recent crown macropodoid phylogenies[12,42–45] and accommodates for fossil taxa that are variously nested along the potoroid and/or macropodid stems[3,9,14,26,46,89–94].

Node dating calibrations

Macropodiformes versus Phalangeriformes split (root node)

Minimimum bound. 27.82 Ma.

Maximum bound. 54.65 Ma.

Explanation. Woodburne *et al.*[95] documented the historically oldest identified macropodiform and phalangeriform fossils, including the informally named "geologically oldest potoroine kangaroo yet known", "*Kyeema mahoneyi*", from the "Winyardiid" interval representing Zone A of the Etadunna Formation in South Australia. Woodburne *et al.*[95] constrained the entire Etadunna Formation sequence to between 25.7–24.2 Ma based on magnetostratigraphic data; however, Metzger and Retallack[54] revised this to between 26.01–23.6 Ma. Megirian *et al.*[96] otherwise specifically constrained their Etadunna Formation Zone A to 25.2–24.9 Ma following the magnetic polarity chrons assigned by Woodburne *et al.*[95]. Murray & Megirian[97] additionally reported isolated teeth attributable to potoroids and possible phalangerids from the upper Oligocene Pwerte Marnte Marnte Local Fauna (LF) in the Northern Territory, which Megirian *et al.*[96] considered to be older than Etadunna Formation Zone A, and thus part of their 'pre-Etadunnan' Australian Land Mammal Age (LMA) series bounded at >30 Ma. Archer *et al.*[98] also listed numerous unequivocal macropodiform and phalangeriform taxa from uppermost Oligocene Zone A deposits of the Riversleigh World Heritage Area in Queensland (see Arena *et al.*[99] for a detailed discussion of the Riversleigh zonation scheme). These included the stratigraphically wide-ranging burramyid, *Burramys brutyi*, which Archer *et al.*[100] delimited with a maximum age of 25 Ma. However, García-Navas *et al.*[13] recalibrated the oldest estimated age of the Riversleigh Faunal Zone A assemblages from the base of the Chattian after Woodhead *et al.*[101], which equates to 27.82 Ma from Cohen *et al.*[102]. We therefore employ this minimum bound for our constrained divergence of Macropodiformes.

Our maximum bound follows Meredith *et al.*[103], who used the radiometric date of 54.6±0.05 Ma from the lower Eocene Tingamarra LF of Murgon in Queensland. This assemblage incorporates the geologically oldest unequivocal australidelphian marsupial fossils[104], and

accommodates for all previous estimates of the earliest split between Macropodiformes and Phalangeriformes within Diprotodontia[5,46,105,106].

Cercartetus versus *Phalanger* split (crown Phalangeroidea)

Minimimum bound. 27.82 Ma.
Maximum bound. 54.65 Ma.
Explanation. The oldest fossil occurrences of *Cercartetus* are currently restricted to lower Pliocene cave deposits[107,108]. However, Brammall & Archer[109] alternatively summarised the much more extensive stratigraphical distribution of its sister taxon *Burramys*, whose oldest fossils include *Burramys wakefieldi*[110], from the uppermost Oligocene Ngama LF representing Etadunna Formation Zone D[96], and *Burramys brutyi*[111] from the Riversleigh Zone A White Hunter Site[98.99]. Archer *et al.*[100] mentioned that the upper Oligocene sequences at Riversleigh might be slightly younger than those in South Australia, but designated a consistent maximum age for both at 25 Ma. Megirian *et al.*[96] otherwise specifically constrained their Etadunna Formation Zone D to 24.1–24 Ma, which postdates the maximum collective limit for the Riversleigh Zone A sites at 27.82 Ma[13].

Similarly, the genus-level affinities of many fossil phalangerids (including *Phalanger*[112]) are historically ambiguous, although the most diagnostic specimens occur in localities correlated with the lower Miocene Riversleigh Zone B[113]. However, Case *et al.*[114] described *Eocuscus sarastamppi* from the Ditjimanka LF, which correlates with the upper Oligocene Etadunna Formation Zone B at "ca. 25 [Ma]", or 24.9–24.6 Ma after Megirian *et al.*[96]. Given these occurrences, we therefore consider the most inclusive minimum bound to be 27.82 Ma based on the oldest estimated age of Riversleigh Faunal Zone A[13]. Our maximum bound alternatively follows Meredith *et al.*[105], who cited the radiometric date of 54.6±0.05 Ma from the lower Eocene Tingamarra LF to most feasibly incorporate the base of Phalangeroidea.

Hypsiprymnodontidae versus Potoroidae + Macropodidae split (crown Macropodoidea)

Minimimum bound. 15.97 Ma.
Maximum bound. 27.82 Ma.
Explanation. Den Boer & Kear[14] established *Palaeopotorous priscus* from the uppermost Oligocene Tarkarooloo LF of the Namba Formation in South Australia as the oldest identifiable stem macropodiform. Woodburne *et al.*[95] correlated the Tarkarooloo LF with Zone D of the laterally equivalent Etadunna Formation. Megirian *et al.*[96] specifically constrained Etadunna Formation Zone D to 24.1–24 Ma. Notably, though, the basally branching macropodoids *Ngamaroo archeri* and *Purtia mosaicus* occur within Etadunna Formation Zone D and Zone C, respectively[84,89]. Following the chronostratigraphic succession of Megirian *et al.*[96], this would suggest a maximum bound of up to 24.6 Ma. However, the phylogenetic relationships of *N. archeri* and *P. mosaicus* are both unresolved[87–94], which is problematic for node dating purposes[44]. Moreover, various putative stem potoroids and macropodids have been described from deposits correlated with the upper Oligocene Riversleigh Zone A[89–94]. All of these taxa are phylogenetically ambiguous leading to uncertainty over their classifications[82]. As a result, we conservatively set our maximum stratigraphic bound for the root split within crown Macropodoidea (= Hypsiprymnodonidae versus Potoroidae + Macropodidae) at 27.82 Ma to accommodate for the recalibrated age of Riversleigh Faunal Zone A after García-Navas *et al.*[13].

The minimum constraint for divergence of Hypsiprymnodontidae (herein restricted to the genus *Hypsiprymnodon*[14,46]) versus Macropodia is delimited by the stratigraphically earliest occurrence of *Hypsiprymnodon*, which has been identified from a range of fossil sites spanning the entire Riversleigh Faunal Zone B[115]; this equates to the lower Miocene B1–B3 intervals of Arena *et al.*[99]. Amongst these, Neville's Garden Site and Camel Sputum Site were both

radiometrically dated by Woodhead *et al.*[101] at 18.24 ± 0.29 and 17.75 ± 0.78, respectively. Nonetheless, Woodhead *et al.*[101] also cited a biocorrelated early Miocene age range of 23–16 Ma for Riversleigh Faunal Zone B, which we recalibrate to 23.03–15.97 Ma using the base of the Aquitanian to top of the Burdigalian after Cohen *et al.*[102]. Our minimum bound for the root divergence within crown Macropodoidea is thus set at 15.97 Ma, which additionally accommodates for many of the youngest mean estimates generated by Brennan[44], Cascini *et al.*[45] and Beck *et al.*[46] using their tip-dating and total evidence dating approaches.

Potoroidae versus Macropodidae split (crown Macropodia)

Minimimum bound. Not designated.
Maximum bound. Not designated.
Explanation. Meredith *et al.*[42] fixed their maximum limit for the split of Potoroidae versus Macropodidae at 24.8 Ma using a stratigraphic bound defined by the absence of demonstrably assignable crown group fossils in Zone A of the Etadunna Formation[95]. This calibration approximates the 24.7 Ma prior employed by Cascini *et al.*[45]. The oldest fossils currently assigned to Potoroidae and/or Macropodidae derive from Riversleigh Zone A[90–94,98], which has been stratigraphically delimited at 27.82 Ma[13]. These include the putative stem potoroid *Gumardee* spp.[94,116], which occurs together with indeterminate "potoroid" fossils in the upper Oligocene Tarkarooloo LF of the Namba Formation[117]. Megirian *et al.*[96] considered the Tarkarooloo LF assemblage 'Pre-Etadunnan', and thus assigned an approximate boundary estimate of >30 Ma. Problematically, however, the various species of *Gumardee* are not recovered as stem potoroids in other phylogenies[88,93,115], and isolated "potoroid" teeth from the Tarkarooloo LF[117] have alternatively been interpreted as stem macropodiforms[14]. Similarly, the informally defined "potoroine", "*Kyeema mahoneyi*", from Etadunna Formation Zone A[95] (if valid) is designated Macropodiformes *incertae sedis*[26]. The historically posited stem potoroids *Purtia mosaicus*[84] from the Ngapakaldi LF of Etadunna Formation Zone C (24.6–24.1 Ma[96]), and *Wakiewakie lawsoni*[50,118] from the Kutjamarpu LF of the Wipajiri Formation in South Australia (= Wipajirian LMA at up to 17.6 Ma[96]) are also phylogenetically unstable[87–94], and have otherwise been referred to the paraphyletic stem macropodid (contra Flannery *et al.*[116]) grouping "Bulungamayinae"[26,46,85,89]. The next oldest stem potoroid, '*Bettongia*' *moyesi*, from the middle Miocene Riversleigh System C Two Trees Site is phylogenetically equivocal[87–94] with some studies even suggesting non-potoroid affinities[26,85]. Furthermore, the age of Two Trees Site has been disputed[119], yet the most recent assessment[99] has reaffirmed its biocorrelation with Riversleigh System C.

The oldest consistently nested crown macropodid fossil[3,9,12,14,26,44,46,49,85,86–94,115,120,121] is the basally branching macropodine, *Dorcopsoides fossilis*, from the upper Miocene Alcoota LF of the Waite Formation in the Northern Territory[122]. Megirian *et al.*[96] radiometrically correlated the Alcoota LF with the Waitean LMA at >5.84 Ma, although competing age estimates have ranged from 7–8 Ma to as much as 12 Ma[9,57,123]. In addition, *Dorcopsoides fossilis* is coeval with the basally branching sthenurine *Hadronomas puckridgi*[122,124], and another uncontested basally branching sthenurine, *Rhizosthenurus flanneryi*, has been recovered from the lower-upper Miocene Riversleigh Zone D Encore Site[121]. Encore Site was estimated at around 12 Ma by Megirian *et al.*[96] and Couzens & Prideaux[9], although Woodhead *et al.*[101] listed a range of 11.6–5.3 Ma, or 11.63–5.333 Ma using the base of the Tortonian to top of the Messinian after Cohen *et al.*[102]. Both Prideaux & Warburton[3] and Couzens & Prideaux[9] phylogenetically bracketed Sthenurinae within crown Macropodidae, as delimited by the basal-most clade incorporating the extant lagostrophine, *Lagostrophus fasciatus*. They also recovered the possible stem macropodid *Wanburoo hilarus* as a sthenurine, although this taxon has been identified from middle Miocene Riversleigh Zone C sites, including Dome Site[124], which Arena *et al.*[98] assigned to the lowermost Riversleigh Zone C1 interval. Woodhead *et al.*[101] considered

Riversleigh Zone C to be between 16–11.6 Ma (with a specific median age of 14.5 Ma allocated for Dome Site by Couzens & Prideaux[9]), or 15.97–11.63 Ma using the base of the Langhian to top of the Serravallian after Cohen *et al.*[102]. Furthermore, Prideaux & Warburton[3] and Couzens & Prideaux[9] included another stem macropodid, *Ganguroo bilamina*, within the paraphyletic basal branches of crown Macropodidae. Prideaux & Warburton[3] derived their original scores for this taxon from undisclosed specimens in the literature, but *G. bilamina* has since been treated as a hypodigm, and split into three species[90,91] that collectively span the Riversleigh zones B–D — an extraordinarily extended age range of 23.03–5.333 Ma. Critically, Brennan[44] found that phylogenetic ambiguity surrounding the placement of *G. bilamina* led to inflated divergence estimates for the Potoroidae-Macropodidae split. Consequently, given these difficulties in pinpointing an alternative more stable age prior, we have elected to leave this node undesignated pending the discovery of more definitively attributable crown potoroid and macropodid fossils.

Potoroinae versus Bettonginae split (crown Potoroidae)

Minimum bound. 3.6 Ma.
Maximum bound. 15.97 Ma.
Explanation. The oldest possible crown potoroine fossils are referred to *Potorous* sp. from the upper Miocene or lower Pliocene Curramulka LF in the Yorke Peninsula of South Australia[126,127]. Megirian *et al.*[96] attributed the Curramulka LF to the Tirarian LMA without detailed explanation, but this provides a geochronometric minimum bound of 3.6 Ma, which equates to the top of the Zanclean after Cohen *et al.*[102]. While the corresponding maximum bound is speculative, we use the base of the Langhian[102] because this is consistent with age priors employed elsewhere[42].

Lagostrophus versus *Simosthenurus* split

Minimum bound. 4.41 Ma.
Maximum bound. 15.97 Ma.
Explanation. The oldest fossils attributed to *Lagostrophus* are late Pliocene to early Pleistocene in age[128], with other possible lagostrophines including the species of *Troposodon* and *Tjukuru wellsi* ranging from the Waitean (>5.84 Ma) to Tirarian LMA[129], with the oldest radiometric date derived from the lower Pliocene Hamilton LF of Victoria[96]. Megirian *et al.*[96] recalibrated the age of the Hamilton LF from Whitelaw[130], which was updated to 4.45±0.04 by García-Navas *et al.*[13] based on the revised C3n.2n age scaling from Gradstein *et al.*[131]. We therefore use this minimum bound for the divergence of Lagostrophinae, which has been recovered in a more basally branching position relative to Sthenurinae by some phylogenies[3,9,132]. Notably, however, our minimum bound also accommodates for the stratigraphically oldest fossils assigned to the genus *Simosthenurus*, which occur in various localities spanning the lower Pliocene to upper Pleistocene[132]. Nevertheless, unequivocal basally branching sthenurines, such as *Rhizosthenurus flanneryi*, are known from the lower-upper Miocene Riversleigh Zone D[98,99], which can be constrained at 11.63 Ma using the base of the Tortonian after Cohen *et al.*[102]. The possible referral of *Wanburoo hilarus* to Sthenurinae[3,9] further extends this range into the middle Miocene Riversleigh Zone C, which we use to delimit our maximum bound of 15.97 Ma from the base of the Langhian[102].

Dorcopsis versus *Dorcopsulus* (crown Dorcopsini)

Minimum bound. 4.41 Ma.
Maximum bound. 11.63 Ma.
Explanation. The stratigraphically earliest uncontested occurrence of the genus *Dorcopsis* is *Dorcopsis wintercookorum* from the lower Pliocene Hamilton LF of Victoria[133]. García-Navas

et al.[13] recalibrated the original radiometric date to a minimum bound of 4.45±0.04. We otherwise set the maximum bound at the base of the Tortonian after Cohen *et al.*[102] because this accommodates for the upper Miocene (>5.84 Ma[96]) *Dorcopsoides fossilis*, which might either be a sister taxon of *Dorcopsis* or more basally branching macropodine[3,9,12,14,26,44,46,49,85,86–94,115,120,121]. Furthermore, our maximum fossil age prior is updated from Meredith *et al.*[42], Dodt *et al.*[80] and Nilsson *et al.*[81], who all used the absence of demonstrable macropodines in Riversleigh Zone C sites at 16–11.6 Ma[101].

Thylogale versus *Petrogale* + *Dendrolagus* split

Minimum bound. 4.41 Ma.
Maximum bound. 11.63 Ma.
Explanation. The minimum bound is set at 4.45±0.04 from the lower Pliocene Hamilton LF[13], which includes *Thylogale ignis*[133]. The maximum bound is designated from the base of the Tortonian after Cohen *et al.*[102], and is delimited by the absence of demonstrable macropodines in Riversleigh Zone C sites[98] at 16–11.6 Ma[101].

Petrogale versus *Dendrolagus* split

Minimum bound. 4.41 Ma.
Maximum bound. 11.63 Ma.
Explanation. Meredith *et al.*[42] set their minimum bound for the divergences of *Petrogale* and *Dendrolagus* at 3.6 Ma representing the latest-early Pliocene; equivalent to the Zanclean-Piacenzian boundary after Cohen *et al.*[102]. Eldridge *et al.*[82] otherwise used 4.46±0.1 to calibrate the *Petrogale-Dendrolagus* split based on the minimum age of the lower Pliocene Hamilton LF[134], which has produced the oldest identified *Dendrolagus* fossils[133]. We accordingly set our minimum bound at 4.45±0.04 based on the Hamilton LF recalibration proposed by García-Navas *et al.*[13].

Eldridge *et al.*[82] cited a maximum "radiometric date" of 14.22 Ma based on the lower–middle Miocene Bullock Creek LF of the Northern Territory[135], and the middle Miocene Riversleigh Zone C[101], which "records no presence of our ingroup taxa". While we agree that no crown group macropodine has yet been identified from strata of this age, we prefer to designate our maximum bound at the base of the Tortonian after Cohen *et al.*[102], which accommodates for the Riversleigh Zone C sites[99] at 16–11.6 Ma[101].

Protemnodon versus *Macropus*+*Notamacropus*+*Osphranter* split

Minimum bound. 4.41 Ma.
Maximum bound. 11.63 Ma.
Explanation. Dawson[107] concluded that the oldest demonstrable occurrence of *Protemnodon* was in the lower Pliocene Hamilton LF. We therefore use this constraint for our minimum bound of 4.45±0.04 following García-Navas *et al.*[13]. Our maximum bound is designated from the base of the Tortonian after Cohen *et al.*[102].

Notamacropus versus *Osphranter* split

Minimum bound. 4.41 Ma.
Maximum bound. 11.63 Ma.
Explanation. Our minimum bound is set at 4.45±0.04 based on the revised C3n.2n age scaling of Gradstein *et al.*[131] for the lower Pliocene Hamilton LF, which includes *Notamacropus* sp.[133]. The maximum bound is designated from the base of the Tortonian after Cohen *et al.*[102].

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