Mammals from 'down under': a multi-gene species-level phylogeny of marsupial mammals (Mammalia, Metatheria)

Laura J May-Collado, C. William Kilpatrick, Ingi Agnarsson

Marsupials or metatherians are a group of mammals that are distinct in giving birth to young at early stages of development and have prolonged investment in lactation. The group consists of nearly 350 extant species, including kangaroos, koala, possums, and their relatives. Marsupials are an old lineage thought to have diverged from early therian mammals some 160 million years ago in the Jurassic, and have a remarkable evolutionary and biogeographical history, with extant species restricted to the Americas, mostly South America, and to Australasia. Although the group has been the subject of decades of phylogenetic research the marsupial tree of life remains controversial, with most studies focusing on only a fraction of the species diversity within the infraclass. Here we present the first Methaterian species-level phylogeny including 80% of the extant marsupial species and five nuclear and five mitochondrial markers obtained from Genbank and a recently published retroposon matrix. Our primary goal is to provide a summary phylogeny that will serve as a tool for comparative research. We evaluate the extent to which the phylogeny recovers current phylogenetic knowledge based on the recovery of "benchmark clades" from prior studies—unambiguously supported key clades and undisputed traditional taxonomic groups. The Bayesian phylogenetic analyses recovered nearly all benchmark clades but failed to find support for the suborder Phalagiformes. The most significant difference with previous published topologies is the support for Australidelphia as a group containing Microbiotheriidae, nested within American marsupials. However, a likelihood ratio test shows that alternative topologies with monophyletic Australidelphia and Ameridelphia are not significantly different than the preferred tree. Although, further data are needed to solidify understanding of Methateria phylogeny, the new phylogenetic hypothesis provided here offers a well resolved and detailed tool for comparative analyses, covering the majority of the known species richness of the group.

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Laura J May-Collado[a,b], C William Kilpatrick[a], & Ingi Agnarsson[a]

[a] *Department of Biology, University of Vermont, 109 Carrigan Drive, Burlington, Vermont 05405*

[b] *Department of Environmental Science and Policy, George Mason University, MSN 5F2, 4400 University Drive, Fairfax VA 22030*

*Corresponding author

E-mail: lmaycollado@gmailcom,

Phone: (802) 656-8646

Introduction

The infraclass Metatheria contains seven mammalian orders that share a reproductive strategy, giving birth to undeveloped young and having prolonged investment in lactation (Aplin & Archer, 1987). The group includes the familiar Australian megafauna, such as kangaroos and koalas, as well as some enigmatic mammals such as wombats, the Tasmanian devil, and the unique South American Monito del Monte. Many species are at risk of extinction (Isaac et al., 2007) and two marsupial families have recently gone extinct, Thylacinidae (1936), and Chaeropodidae (~1950). Marsupials have a rather unusual geographic distribution mostly inhabiting Australasia and South America (Nilsson et al., 2004), with a few genera having relatively recently crossed the Panamanian isthmus and one species reached northern North America, the Virginia opossum. Most prior phylogenetic work has suggested that marsupials colonized Australia twice via Antarctica/South America during the breakup of Gondwanaland (Nilsson et al., 2004). However, a recent study supports the monophyly of the Australasian marsupials and thus that marsupials reached Australasia in a single migration event (Nilsson et al., 2010), and then diversified with over 200 extant species in the region.

Marsupials are typically classified into two major cohorts the Australidelphia and the Ameridelphia (Aplin & Archer, 1987; Marshall et al., 1990) based in part on differences of the ankle joints (Szalay, 1982). Australidelphia consists of five orders, Dasyuromorphia (i.e., carnivorous marsupials, marsupial mice), Peramelemorphia (i.e., bilbies, bandicoots), Notoryctemorphia (marsupial moles), Diprotodontia (i.e., koalas, wombats, kangaroos, possums), and the South American order Microbiotheria (monito del monte). Ameridelphia consist of two orders Didelphimorphia (opossums) and Paucituberculata (shrew oposums), mainly distributed in South America (Gardner, 2005a-b). Most recent studies, however, have shown that Ameridelphia is non-monophyletic and thus this classification is inconsistent with phylogenetic knowledge (Horovitz & Sánchez-Villagra, 2003; Nilsson et al., 2010).

While the phylogenetics of marsupials has received much attention in recent years, the marsupial tree of life remains incompletely resolved (Nilsson et al., 2010). Most studies focus on solving phylogenetic relationships within orders (Krajewski et al., 1997; Blacket et al., 1999; Jansa et al., 2006; Meredith et al., 2008; Frankham et al., 2012), while the root of the marsupial tree and the relationships among the four Australasian and three South American marsupial orders have not been resolved conclusively with standard sequence data, or morphological evidence (Springer et al., 1998; Horovitz & Sánchez-Villagra, 2003; Nilsson et al., 2003; Asher et al., 2004; Nilsson et al., 2010). Particularly contentious has been the early branching pattern within Metatheria. For example, it is unclear whether Paucituberculata or Didelphimorphia are the sister group to the remaining marsupials (Meredith et al., 2009). Furthermore, the phylogenetic position of the enigmatic Microbiotheria, represented by only one American species 'monito del monte' (*Dromiciops gliroides*), differs among studies (Springer et al., 1998; Burk et al., 1999; Amrine-Madsen et al., 2003; Nilsson et al., 2003, 2004), but is usually placed among the Australasian marsupials, implying a biogeographical history that is not straightforward to interpret. However, Nilsson et al., (2010) provided an analysis of retroposon insertions that provide a powerful alternative to sequence data, especially to resolve deeper level relationships. They find support for an intuitively pleasing hypothesis placing all Australasian marsupials in a single clade, as a sister group to Microbiotheria. They also provide strong evidence that Didelphimorphia forms the sister group of the remaining marsupials. A few other studies have studied marsupial species-level relationships mainly within small taxonomic groups (families and subfamilies, genera) or employing relatively sparse taxon sampling (Krajewski et al., 1997, 2012; Blacket et al., 1999; Jansa et al., 2006; Meredith et al., 2008; Malekian et al., 2010; Frankham et al., 2012).

Detailed species-level phylogenies underlie modern comparative studies (Harvey & Pagel, 1991). In general, statistical power of comparative methods increases as taxon sampling and resolution improves. In addition, many methods in the toolkit of comparative biology perform best when branch length estimates are available (Felsenstein, 2004; Bollback, 2006). At present the most detailed species-level phylogeny of marsupials available is based on a supertree including approximately 260 taxa (Cardillo et al., 2004). This phylogeny has already proven to be a high utility tool, underlying various comparative analyses (Weisbecker et al., 2008; Sánchez-Villagra et al., 2008; Flores et al., 2013). Yet, supertrees are constrained by the available input data, often lacking full resolution and typically without accurate estimates of branch lengths. Here we present a species-level phylogeny with branch-length information, including 276 marsupial species, with the primary goal of providing an additional tool for taxonomy, phylogenetic estimation of conservation priorities, and comparative hypothesis testing. We evaluate the 'reliability' of the phylogeny based on the recovery of numerous benchmark clades-previously supported clades and undisputed taxonomic groups (Agnarsson & May-Collado, 2008).

Material and Methods

Data and phylogenetic analyses

Sequences for five mitochondrial genes (Cyt b, 12S and 16 S rRNA, COI, NADH2) and five nuclear genes (ApoB, IRBP, Rag1, BRCA1, and protamine) were downloaded for 271 extant marsupial taxa and five extinct species (Table S1). When different genes were available for different subspecies we fused the data to represent the species. We selected 19 outgroups species representing the diversity of Mammalia, including the orders Monotremata, Pilosa, Pholidota, Chiroptera, Rodentia, Dermoptera, Carnivora, Erinaceomorpha, Soricomorpha, Scandentia, Perissodactyla and the supraorder Cetartiodactyla and the magnaorder Afrotheria. We created several data partitions for sensitivity analyses to explore potential data conflict and source of support, or lack of support for the phylogeny. Conflict and lack of support might be expected because 1) mitochondrial and nuclear genes often differ in estimation of deeper level clades, 2) we identified some alignment issues in the protamine data suggesting potential problems with the source sequences, and 3) the data come from Genbank and are fragmentary in that most species are missing part of the character data, with some species having less than 10% data coverage. These partitions consisted of the following five matrices: mtDNA (282 taxa), nuDNA (243 taxa), nuDNA excluding protamine (237 taxa), all data concatenated (296 taxa), all data minus protamine (296 taxa), and the full concatenated character matrix, but excluding species with less than 10% data coverage (251 taxa). Finally, we added the retroposon matrix of Nilsson et al., (2010) to the full and the 251 taxon matrices as a partition. Sequences were downloaded via Mesquite 275v (Maddison & Maddison, 2011), aligned in Mafft (http://wwwebiacuk/) and then reintroduced to Mesquite and manually inspected.

The appropriate models for the Bayesian analysis were selected with jModeltest (Darriba et al.,~~;~~ 2012) using the AIC criterion (Posada & Buckley, 2004) with a UPGMA tree chosen as the basis for Modeltest. The selected models of sequence evolution for Cyt b, 12S, 16S, ApoB, IRBP, RAG1, NADH2, and BRCA1was GTR+I+G, and for COI and Protamine1 the selected models were HKY+I+G and HKY+G, respectively. The retroposon partition was analyzed under a parsimony model, and alternatively using GTR+I+G. Bayesian analyses were performed through the CIPRES Science Gateway the maximum time offered by that server (167 hours) using the hybrid version (CIPRES Science Gateway v33) of MrBayes 312 (Huelsenbeck &

Ronquist, 2001; Ronquist & Huelsenbeck, 2003) with settings as in May-Collado & Agnarsson (2006) and Agnarsson & May-Collado (2008) with separate model estimation each gene and within protein coding genes for first, second, and third codon positions. The Markov chain Monte Carlo search for each matrix ran with four chains for approximately 18,000,000 sampling the Markov chain every 1000 generations, and the sample points of the first 5,000000 generations were discarded as 'burnin', after which the chains had reached approximate stationarity.

As the basal topology of our preferred tree differs from many recent studies, we performed a Shimodaira-Hasawa test (Shimodaira & Hasawa, 1999) to see if alternative topologies could be rejected. We tested topologies where 1) Didelphimorphia is sister to the remaining Metatheria, 2) where Ameridelphia is monophyletic, and 3) where Australian Australidelphia is monophyletic (that is Microbiotheriidae is sister to other Australidelphia) The test was run in PAUP* (Swofford, 2003) under a GTR+I+G model.

Benchmark clades

An intuitive approach of evaluating if the species-level phylogeny will serve, as a reliable comparative tool is the recovery well supported clades from prior studies and undisputed taxonomic groups (May-Collado & Agnarsson, 2006; Agnarsson & May-Collado, 2008). This approach is valuable given the nature of this study. The matrix is based on available data from Genbank rather than markers chosen specifically for the question at hand, some may not be ideal to estimate ancient phylogenetic relationships, and many data are missing in the concatenated matrix. The recovery of benchmark clades is a simple 'reality check', indicating that the analyses are not critically impeded by these shortcomings of the data. The following list of benchmark clades is meant to be representative not exhaustive, focusing on deeper clades that have been supported by multiple studies.

Australidephia: This superorder contains all Australian marsupials and a single species from South America: Kirsch et al., (1991) (DNA hybridization), Colgan (1999) (Phosphoglycerate Kinase DNA sequences), Palma & Spotorno (1999) (rRNA 12S), Amrine-Madsen et al., (2003) (nuclear DNA), Horovitz & Sánchez-Villagra (2003) (postcranial morphology, dental, and soft tissue), Asher et al., 2004 (osteological, dental, and soft tissue, nuclear and mitochondrial DNA), Baker et al., (2004) (RAG1), Carrillo et al., (2004) (supertree), Nilsson et al., (2004) (mtDNA, complete genomes), Phillips et al., (2006) (mtDNA, nuDNA), Beck (2008) (molecular supermatrix), Meredith et al., (2008, 2009) (nuDNA), Ladèveze & de Muizon (2010) (morphology, fossils), Nilsson et al., (2010) (retroposon insertions), Westerman et al., (2010) (nuDNA).

Diprotodontia: This is the largest order of marsupials and is distinguished from other marsupials by having syndactylous digits and two procumbent lower incisors (diprotodont): Baverstock et al., (1990) (albumin immunology), Burk et al., (1999) (mtDNA), Colgan (1999) (Phosphoglycerate Kinase DNA sequences), Osborne et al., (2002) (mtDNA), Amrine-Madsen et al., (2003) (nuclear DNA), Horovitz & Sánchez-Villagra (2003) (postcranial morphology, dental, and soft tissue), Asher et al., 2004 (osteological, dental, and soft tissue, nuclear and mitochondrial DNA), Carrillo et al., (2004) (supertree), Phillips et al., (2006) (mtDNA, nuDNA), Meredith et al., (2008, 2009) (nuDNA), Munemasa et al., (2008) (CORE-SINE), Phillips & Pratt (2008) (mtRNA, mtDNA, nuDNA), Nilsson et al., (2010) (retroposon insertions), Westerman et al., (2010) (nuDNA).

Phalangeriformes: This suborder of Diprotodontia contains medium sized arboreal marsupials from Australia, New Guinea and Sulawesi: Flannery (1987) (morphology), Springer & Woodburke (1989) (basicranial morphology), Springer & Kirsch (1989, 1991) (DNA-hybridization), Amrine-Madsen et al., (2003) (nuDNA), Phillips & Pratt (2008) (mtRNA, mtDNA, nuDNA), Meredith et al., (2009) (nuDNA).

Phalangeroidea: This superfamily of Phalangeriformes contains two families Phalangeridae and Burramyidae: Colgan (1999) (Phosphoglycerate Kinase DNA sequences), Meredith et al., (2008) (nuDNA), Westerman et al., (2010) (nuDNA).

Phalangeridae: This family of Phalangeroidea contains brushtail possums and cuscuses: Hughes (1965) (sperm morphology), Osborne et al., (2002) (mtDNA), Baker et al., (2004) (RAG1), Carrillo et al., (2004) (supertree), Kavanagh et al., (2004) (mtDNA), Raterman et al., (2006) (nuDNA), Beck (2008) (molecular supermatrix), Meredith et al., (2009) (nuDNA).

Burramyidae: This family of Phalangeroidea contains pygmy possums: Archer (1984) (morphology), Barverstock et al., (1990) (albumin immunology), Edwards & Westerman (1995) (DNA-DNA hybrization), Osborne & Christidis (2002) (ND2 gene), Carrillo et al., (2004) (supertree), Beck (2008) (molecular supermatrix).

Petauroidea: This superfamily of Phalangeriformes contains four families, Pseudocheiridae, Petauridae, Tarsipedidae, and Acrobatidae: Osborne et al., (2002) (mtDNA), Amrine-Madsen et al., (2003) (nuDNA), Kavanagh et al., 2004 (mtDNA), Meredith et al., (2008) (nuDNA), Phillips

& Pratt (2008) (mtRNA, mtDNA, nuDNA), Meredith et al., (2010) (nuDNA), Westerman et al.,, (2010) (nuDNA).

Pseudocheiridae: This family of the superfamily Petauroidea contains ringtail possums: Hayman & Martin (1974) (karyology), Archer (1984) (cranio-dental morphology), Barverstock et al., (1990) (albumin immunology), Westerman et al., (1990) (DNA-DNA hybridization), Springer (1993) (cranio-dental morphology), Baverstock et al., (1999), (microcomplent fixation), Osborne & Christidis (2001), Carrillo et al., (2004) (supertree), Meredith et al., (2010) (nuDNA).

Petauridae: This family of the superfamily Petauroidea contains gliders, Leadbeater's possum, and the striped possum and trioks: Kirsch & Calaby (1977) (DNA-DNA hybrization), Aplin & Archer (1987), and Smith (1984) (morphology), McKay (1984) (chromosomal characters), Barverstock et al., (1990) (albumin immunology), Osborne & Christidis (2001) (mtDNA), Meredith et al., (2010) (nuDNA).

Acrobatidae: This family of the superfamily Petauroidea contains feather-tailed gliders and feather-tailed possum: Barverstock et al., (1990) (albumin immunology), Baker et al., (2004) (RAG1).

Macropodiformes: This suborder of Diprotodontia contains kangaroos, wallabies, and allies (bettongs, potaroos, and rat kangaroos): Ride (1961), Case (1984), Flannery (1987) (morphology), Kirsch (1977) (serology), Burk & Springer (2000) (mtDNA and nuDNA), Kavanagh et al., (2004) (mtDNA), Meredith et al., (2008, 2009) (nuDNA), Westerman et al.,,

(2010) (nuDNA).

Macropodoidea: This superfamily of Macropodiformes consists of two families the Macropodidae and Potoroidae that form a clade distinct from the rat kangaroo, family Hypsiprymnodontidae: Barverstock et al., (1990) (albumin immunology), Burk et al., (1998) (mtDNA), Colgan (1999) (Phosphoglycerate Kinase DNA sequences), Osborne et al., (2002) (mtDNA), Meredith et al., (2008b) (nuDNA), Phillips & Pratt (2008) (mtRNA, mtDNA, nuDNA).

Macropodidae: This family of Macropodoidea contains the major diversity of marsupial hervibores, including kangaroos, wallabies, tree-kangaroos and several others: Barverstock et al., (1990) (albumin immunology), Burk et al., (1998) (mtDNA), Horovitz & Sánchez-Villagra (2003) (postcranial morphology, dental, and soft tissue), Baker et al., (2004) (RAG1), Carrillo et al.,, (2004) (supertree), Kavanagh et al., 2004 (mtDNA), Meredith et al., (2008b) (nuDNA), Meredith et al., (2009) (nuDNA), Prideaux & Warburton (2010) (osteology).

Potoridae: This family of Macropodoidea contains bettongs: Archer (1984) (morphology), Flannery et al., (1984) (morphology), Flannery 1989 (morphology), Barverstock et al., (1990) (albumin immunology), Sanclair & Westerman (1997) (allozyme electrophoresis and cytb), Burk et al., (1998) (mtDNA), Meredith et al., (2008b) (nuDNA).

Vombatiformes: This suborder of Diprotodontia consists of two families Phascolarctidae and Vombatidae: Hughes (1965) (sperm morphology), Barverstock et al., (1990) (albumin immunology), Burk et al., (1999) (mtDNA), Osborne et al., (2002) (mtDNA), Amrine-Madsen et al., (2003) (nuDNA), Baker et al., (2004) (RAG1), Kavanagh et al., 2004 (mtDNA), Meredith et al., (2008, 2009) (nuDNA), Phillips & Pratt (2008) (mtRNA, mtDNA, nuDNA), Westerman et al., (2010) (nuDNA).

Dasyuromorphia: This order of marsupials contains most of Australian carnivorous marsupials consisting of three families Dasyuridae, Myrmecobiidae, and Thylacinidae: Burk et al., (1999) (mtDNA), Wroe et al., (2000) (cranial and dental morphology), Amrine-Madsen et al., (2003) (nuDNA), Horovitz & Sánchez-Villagra (2003) (postcranial morphology, dental, and soft tissue), Carrillo et al., (2004) (supertree), Kavanagh et al., 2004 (mtDNA), Beck (2008) (molecular supermatrix), Meredith et al., (2009) (nuDNA), Ladèveze & de Muizon (2010) (morphology, fossils), Nilsson et al., (2010) (retroposon insertions), Westerman et al., (2010) (nuDNA).

Dasyuridae: This family of Dasyuromorphia consists of terrestrial and arboreal species, many of which lack a pouch: Westerman & Woolley (1990) (karyotypes), Colgan (1999) (Phosphoglycerate Kinase DNA sequences), Wroe et al., (2000) (cranial and dental morphology), Baker et al., (2004) (RAG1), Carrillo et al., (2004) (supertree), Asher & Kirsch (2006) (morphology), Meredith et al., (2008) (nuDNA).

Notoryctemorphia: This order of marsupials contains two species of marsupial moles, *Notoryctes caurinus* and *N typhlops*: Aplin & Archer (1987) (morphology), Barverstock et al., (1990) (albumin immunology), Springer et al., (1998), Nilsson et al., (2010) (retroposon insertions), Archer et al., (2011) (fossils).

Peramelemorphia: This order of marsupials consists of three families Peramelidae, Chaeropodidae and Thylacomidae: Burk et al., (1999) (mtDNA), Wroe et al., (2000) (cranial and dental morphology), Amrine-Madsen et al., (2003) (nuDNA), Horovitz & Sánchez-Villagra (2003) (postcranial morphology, dental, and soft tissue), Asher et al., 2004 (osteological, dental, and soft tissue, nuclear and mitochondrial DNA), Baker et al., (2004) (RAG1), Kavanagh et al., 2004 (mtDNA), Beck (2008) (molecular supermatrix), Meredith et al., (2008, 2009) (nuclear DNA), Ladèveze & de Muizon (2010) (morphology, fossils), Nilsson et al., (2010) (retroposon insertions), Westerman et al., (2010, 2012) (nuDNA, mtDNA).

Peramelidae: This family of the Peramelemorphia contains bandicoots and echymiperas: Phillips et al., (2006) (mtDNA, nuDNA), Meredith et al., (2008) (nuDNA), Westerman et al., (2012) (nuDNA, mtDNA).

Paucituberculata: This order of shrew opossums is represented by a single family Caenolestidae: Marshall (1980) (morphology, fossils), Sánchez-Villagra (2001), Amrine-Madsen et al., (2003) (nuclear DNA), Carrillo et al., (2004) (supertree), Nilsson et al., (2004) (mtDNA, complete genomes), Asher & Kirsch (2006) (morphology), Phillips et al., (2006) (mtDNA, nuDNA), Meredith et al., (2008) (nuDNA), Ladèveze & de Muizon (2010) (morphology, fossils), Nilsson et al., (2010) (retroposon insertions), Abello (2013) (morphology, fossils).

Didelphimorphia: This order of new world marsupials diversified mainly in South America and consists of a single family Didelphidae: Burk et al., (1999) (mtDNA), Amrine-Madsen et al., (2003) (nuclear DNA), Horovitz & Sánchez-Villagra (2003) (postcranial morphology, dental, and soft tissue), Baker et al., (2004) (RAG1), Carrillo et al., (2004) (supertree), Kavanagh et al., 2004 (mtDNA), Nilsson et al., (2004) (mtDNA, complete genomes), Asher & Kirsch (2006) (morphology), Phillips et al., (2006) (mtDNA, nuDNA), Meredith et al., (2008) (nuDNA) Ladèveze & de Muizon (2010) (morphology, fossils), Nilsson et al., (2010) (retroposon insertions).

Results

Benchmark clades

With the exception of Phalangeriformes data partitions in general supported the majority of benchmark clades (Fig. 1). There were notable differences between the mtDNA partition and the remaining partitions. The mitochondrial data alone resulted in a phylogenetic hypothesis in greater conflict with taxonomy and recent phylogenetic studies than did combined analyses, and analyses of nuclear data alone, particularly at lower taxonomic levels (Figs. 1, S1-S4). In the analysis of all data combined, many clades have low support and some species appear conspicuously misplaced, such as *Marmosa tyleriana* (Fig. S1). Excluding protamine from the nuclear partition in general resulted in the same relationships (Fig. S2). Removing from the concatenated analysis taxa with <10% character data in general recovered the majority of benchmark clades as the full analysis, while support for many clades increased and no species are conspicuously misplaced (Fig. 2-4).

Phylogenetic relationships among orders

The deeper level phylogenetic relationships among orders were consistent between analyses (Fig. 1). These analyses did not support the monophyly of Ameridelphia but instead placed

Paucituberculata as the lineage sister to the remaining marsupials, composed of Didelphimorphia plus Australidelphia. In addition, our results did not support the clade Eometatheria as defined by Kirsch et al., (1997), a clade consisting of Microbiotherida and all autralidelphians excluding peramelians, or as defined by McKenna & Bell (1997), a clade consisting of the Dasuromorphia, Notorycterimorphia, Peramelemorphia, Diprotodontia but excluding Microbiotherida. The South American Microbiotheria was the sister lineage of Diprotodontia in all analyses, though this relationship was never strongly supported. The relationship between Dasyuromorphia and Peramelemorphia to the exclusion of Notorycterimorphia was moderately supported by some of the analyses (Fig.1). These results do not support a sister group relationship between the Peramelemorphia and the Diprotodontia expected of the two members of the Grandorder Syndactyli proposed by McKenna & Bell (1997).

Phylogenetic relationships within orders

~~*Orders*~~ *Paucituberculata:* Within Paucituberculata, the genus *Caenolestes* was sister to a clade containing *Lestoros* and *Rhyncholestes* was strongly supported in the all concatenated (92) and mtDNA analyses (926) (Figs. S1, S4).

Didelphimorphia: Within Didelphimorphia, the monophyly of subfamily Caluromyinae as recognized by Voss and Jansa (2009) containing *Caluromys* and *Caluromysiops* was supported (Figs. S1-S3). The only extant species of the subfamily Hyladelphinae (Voss & Jansa, 2009), the Kalinowski’s mouse opossum (*Hyladelphys kalinowskii*) was sister to Didelphinae and the only member of the subfamily Glironiinae the bushy-tailed opossum, *Glironia venusta* was placed in most analyses basal to the clade containing the Hyladelphinae and the Didelphinae (Figs. 2, S1-

S3). The monophyly of the subfamily Didelphinae (*Marmosa, Monodelphis, Tlacuatzin, Metachirus, Chironectes, Didelphis, Lutreolina, Philander and Chacodelphys, Cryptonanus, Gracilinanus, Lestodelphys, Marmosops*, and *Thylamys*) was supported by the all analyses except the mtDNA partition (Fig. S4). The monophyly of the genera *Thylamys*, *Cryptonatus*, *Marmosops*, *Monodelphis*, *Gracilinanus*, *Philander* and *Marmosa* (plus subgenus *Micoureus)* were supported by most analyses (Figs. 2, S1-S4). Other interesting relationships include the sister relationship of the genera *Cryptonanus* and *Gracilinanus*, the placement of the grayish mouse opossum (*Tlacuatzin canescens*) sister to *Marmosa,* and the close relationship of the Patagonian opossum (*Lestodelphys halli*) with members of the genus *Thylamys*, and the basal position of the Virginia opossum (*Didelphis virginiana*) to *Philander* and the other taxa of *Didelphis* (Figs. 2, S1-S4).

~~Order~~ Peramelemorphia: Within Peramelemorphia the monophyly of the family Peramelidae was corroborated in two of the five analyses (Fig.1). The extinct member of the family Chaeropodidae the pig-footed bandicoot (*Chaeropus ecaudatus*) was placed as sister to the remaining peramelemorphians in the full multigene and mtDNA analyses with strong support (Figs. S1, S4). In contrasts, it was placed in the nuDNA analyses within the Peramelidae either sister to Echymiperinae (Fig. 3) or within the Microperoryctes (Figs. S2-S3, but the support for these placements was low (Figs.3, S2-S3). In three of the analyses the greater bilby, *Macrotis lagotis* (Thylacomyidae) was placed as sister to the remaining peramelemorphians (Fig. 3, S1, S4). The monophyly of the genera *Echymipera, Peroryctes*, *Isoodon* and *Perameles* was supported by all analyses. The multigene analyses differed from the mtDNA partition in the relations among genera. The most supported relationship was ((*Echymipera + Microperorycte*s)

Peroryctes)+ (*Isoodon* + *Perameles*)) (Figs. 3, S1).

~~*Order*~~ *Dasyuromorphia:* Within Dasyuromorphia the monophyly of the families Dasyuridae, Myrmecobiidae, and Thylacinidae was supported by most analyses with the interrelationships (Thylacinidae (Myermecobiidae, Dasyuridae)) (Fig1). In two analyses the extinct Tasmanian wolf (*Thylacinus cynocephalus*) was placed within the subfamily Dasyurinae, but this relationship was poorly supported (Figs. S2, S3). The monophyly of the two Dasyuridae subfamilies Dasyurinae and Sminthopsinae was also supported by most analyses, as well as the two Dasyurinae tribes Dasyurini and Phascogalini and the two Sminthopsinae tribes Planigalini and Sminthopsini (Figs. 3, S1-S4). In all the analyses the phylogenetic position of *Ningaui* and the Kultarr (*Antechinomys laniger*) rendered the genus *Sminthopsis* paraphyletic (Figs.3, S1-S4). Also highly consistent across analyses is the phylogenetic position of the Mulgara (*Dasycercus cristicauda*) as sister to the Ningbing fals antechinus, *Pseudantechinus ningbing* also rendering the later paraphyletic (Figs. 3, S1-S4). The monophyly of the genera *Planigale*, *Murexia*, *Antechinus*, *Phascogale*, *Myoictis*, and *Dasyurus* was supported by most analyses (Figs. 3, S1-S4).

~~*Order*~~ *Diprotodontia*: With the exception of Phalangeriformes the monophyly of other suborders, Vombatiformes (Vombatidae + Phascolarctidae) and Macropodiformes (Hypsiprymnodontidae + Macropodidae + Potoroidea) were supported by all analyses. The monophyly of superfamily Macropodidea was also supported (Fig. 1). The position of Vombatiformes as sister to the remaining Diprododontia was supported by all analyses except the mtDNA partition (Figs. 4, S1-S4). Within the superfamily Petauroidea (Acrobatidae, Tarsipedidae, Pseudocheiriidae, and

Petauridae) all partitions except mtDNA supported the position of the family Acrobatidae as the basal petauroids (Figs. 4, S1-S3). Tarsipedidae was sister to a clade containing Petauridae and Pseudocheiridae. Within Petauridae, the placement of the *Gymnobelideus* sister to the genus *Dactylopsila* to the exclusion of *Petaurus* was strongly supported by both multigene and the mtDNA partitions (Figs. 4, S1-S4). The monophyly of the Pseudocheiridae was strongly supported as well as that of the subfamilies Pseudocheirinae (*Pseudochirulus*, *Pseudocheirus*), Hemibelidinae (*Hemibelideus*, *Petauroides*) and Pseudochiropsinae (*Petropseudes*, *Pseudochirops*). The monophyly of genus *Pseudochirops* was not supported is some of the analyses (Figs. 4, S1-S4). The superfamily Petauroidea is the sister group of Macropodiformes, and Phalangeroidea is the sister group to the clade containing Phalangeridae and Burramyidae (Fig.1). Within the Phalangeridae, the Sulawesi cuscus (*Strigocuscus celebensis*) was the sister species of the Sulawesi bear (*Ailurops ursinus*) in all partitions, rendering the genus *Strigocuscus* paraphyletic and the scaly tailed opossum *Wyulda squamicaudata* was sister to *Trichosurus* (Figs. 4, S1-S4). The three kangoroo families Potoroidea, Macropodidae, and Hypsiprymnodontidae were monophyletic and their interrelationships Hypsiprymnodontidae (Potoroidea (Macropodidae) were also supported by all partitions except mtDNA (Figs.1, S1-S4). Within Potoroidea the Rofous bettong, *Aepyprymnus* rufescens was sister to *Bettongia* to the exclusion of *Potorous* (Figs.4, S1-S3). Within Macropodidae, the monophyly of *Macropus* and *Petrogale*, was not supported by all analyses (Figs.4, S1-S3). In the full multigene analysis Bennett's tree kangaroo, *Dendrolagus bennettianus* was the basal species in macropodids (Fig. S1), but for the pruned multigene analysis and nuclear analyses it was instead sister to all *Phalanger* species (Figs. 4, S2-S3).

Discussion

Our phylogenetic analyses include the largest taxon sampling of extant marsupial species to date, accounting for approximately 80% of the marsupial diversity currently recognized in Mammal Species of the World (Wilson & Reeder, 2005) or by the 2012 IUCN Red List of Threatened Species (http://wwwiucnredlistorg/). Overall, analyses resulted in phylogenetic trees congruent among partitions and in agreement with current phylogenetic knowledge, other than the basal arrangement of the American orders (see below). The mtDNA partitions resulted in deeper level relationships that are inconsistent with existing knowledge, thus the mtDNA data seems to be partially misleading, especially regarding deeper level relationships (Nilsson et al., 2010). However, the mtDNA data corroborates most of the shallower clades, and nuclear data signal appears to trump the mtDNA data in the combined analysis, resulting in hypotheses mostly consistent with taxonomy and current phylogenetic understanding. Missing data~~,~~ and ambiguity in the protamine data ~~does~~ not seem to have strong impact on the analysis, but these factors in part explain low support for various clades in the full dataset analysis. Support in analyses excluding species with most missing data was higher for many clades (Figs. 2-4). This finding is not surprising, it is well documented that placement of taxa with excessive (>90%) missing data can be problematic.

Root of the Marsupial tree

As in most recent phylogenetic analyses, our result fails to support a monophyletic Ameridelphia, a clade only supported by mtDNA in this study and a few other studies (Retief et al., 1995; Springer et al., 1994, 1998). All analyses resulted in the same basal arrangement, (Paucituberculata (Didelphimorphia, Australidelphia)). This conflicts with prior analyses in that the placement of Paucituberculata and Didelphimorphia are switched (Carrillo et al., 2004, Nilsson et al., 2010). However, a likelihood ratio test demonstrated that the alternative topologies consistent with recent studies are not significantly ‘worse’ explanations of the sequence data. For example, topologies consistent with the retroposon study of Nilsson et al., (2010) and topologies with monophyletic Ameridelphia have only slightly lower likelihoods than the preferred tree (see Table 1). Amrine-Madsen et al.~~,~~ (2003) also could not discriminate between these hypotheses, but favored rooting at the base of Didelphimorphia due to the long-branch leading to caenolestids. The sister relationship of Paucituberculata to Australasian marsupials is also supported by morphological characters (Horovitz & Sánchez-Villagra, 2003) and molecular studies (Baker et al., 2004; Kirsch, 1977; Kirsch et al., 1997).

Phylogenetic relationships between orders

Our results further place Microbiotheriidae within Australidelphia sister to Diprotodontia, and this relationship is supported independently by the mtDNA and nuDNA. This result is congruent with a previous morphological study that suggested *Dromiciops* and diprotodontians shared a common ancestor that was hypothesized to have had a prehensile tail and an opposable hallux (Horovitz & Sánchez-Villagra, 2003). These taxa also share sperm morphology (Gallardo & Patterson, 1987) and have been supported previously using sequence data (Kirsch et al., 1991), and in a supertrees analysis (Carrillo et al., 2004). However, this arrangement is in conflict with some recent molecular studies (Asher et al., 2004; Beck, 2008; Nilsson et al., 2010). In particular, the recent retroposon analysis of Nilsson et al.~~,~~ (2010) Microbiotheriidae is strongly supported as sister to the Australasian Australidelphia. The latter hypothesis is more congruent with the geographical distribution, placing all Australasian species in a clade and suggesting a single origin of Australasian marsupials. The source of this conflict between sequence and retroposon data is unclear. Future work should ~~profitably~~ focus on adding single-copy nuclear markers, retroposon data for a larger taxon sample, and ultimately employing rich sources of data through next generation sequencing techniques to solidify Marsupialia phylogenetics.

We find a moderately supported relationship between the orders Peramelemorphia and Dasyuromorphia to the exclusion of Notorycterimorphia, similar to previous studies (Baverstock et al., 1990; Kullander et al., 1997; Springer et al., 1997; Beck, 2008; Colgan, 1999; Nilsson et al., 2010). The placement of *Notoryctes*, has been controversial, with several studies supporting the genus as sister to Dasyuromorphia (Amrine-Mandsen et al., 2003; Burk et al., 1999; Springer et al., 1998) while others as sister to Peramelemorphia (Baker et al., 2004). Finally, we did not find support for the Eometatheria as contra a prior study combining molecular and morphological data (Asher et al., 2004).

Phylogenetic relationships within orders

~~Orders~~ Paucituberculata and *Didelphimorphia***:** As found by recent molecular and morphological studies the two Ameridelphian orders Paucituberculata and Didelphimorphia form a grade rather than a clade (Horovitz & Sánchez-Villagra, 2003; Nilsson et al., 2010). However, we cannot reject the monophyly of Ameridelphia. Our results corroborate Voss and Jansa’s (2009) combined morphological and molecular study where *Glirona* is placed in a monotypic subfamiliy Glironiinae, whereas *Caluromys* and *Caluromysiops* remained in the subfamily Caluromyninae. In addition, *Hyladelphys* was not grouped with other didelphids, thus we support its placement within a subfamily of its own Hyaldephinae (Voss & Jansa, 2009). However, not all analyses supported Didelphinae tribes (Marmosini, Metachirini, Didelphini, and Thylamyini). The species

of the genus *Cryptonanus* (Voss et al., 2005) were formerly included in the genus *Gracilinanus* by Gardner & Creighton (1989). Previous studies have found it difficult to establish the phylogenectic position of *Cryptonanus* (Jansa et al., 2006; Flores, 2009), however, with the exception of the mtDNA analyses we found strong support for a sister relationship between *Cryptonanus* and *Gracilinanus.* A close phylogenetic position of the greyish mouse opossum, *Tlacuatzin canescens* (formely *Marmosa canescens*) with species of the genus *Marmosa* (and subgenus *Micoureus*) is also supported, as has been found in previous studies combining molecules and morphology (Flores, 2009; Voss & Jansa, 2009). The basal placement of the Peruvian gracile mouse opossum, *Hyladelphys kalinowskii* in the Didelphinae is also supported by morphological and molecular analyses (Flores, 2009), as well as the close phylogenetic relationship between Patagonian opossum, *Lestodelphys halli* and the genus *Thylamys* (Carillo et al., 2004; Jansa et al., 2006; Flores, 2009).

~~Orders~~ Dasyuromorphia: Our results for Dasyuromorphia are largely congruent with previous studies in supporting the monophyly of dasyuromorphian families and subfamilies. The phylogenetic position of the Numbat (*Myrmecobius fasciatus*) and the Tasmanian wolf (*Thylacinus cynocephalus*) are traditionally controversial. In this study, most analyses strongly supported the basal placement of the Tasmanian wolf and the Numbat to all dasyuromorphians. This phylogenetic arrangement has been previously supported by morphological (Wroe et al., 2000) and combined analyses (Beck, 2008) including a supertree analysis (Carillo et al., 2004). The position of the *Ningaui* and the Kultarr (*Antechinomys laniger*) within *Sminthopsis* agrees with previous studies (Archer, 1975; Blacket et al., 1999; Carillo et al., 2004) and suggests that taxonomic changes are necessary to accommodate this phylogenetic perspective, In the supertree

analysis by Carillo et al., (2004) the Kultarr was referred as *Sminthopsis laniger* and it was placed as basal to a clade containing *Ningaui* and *Sminthopsis* species. Also highly consistent across analyses in this study is the phylogenetic position of the Mulgara, *Dasycercus cristicauda* as sister to the Ningbing false antechinus, *Pseudantechinus ningbing* contrasting the results by Carillo et al., (2004).

~~Order~~ Peramelemorphia: The results from the complete multigene and mtDNA data sets are congruent with recent studies placing the extinct pig-footed bandicoot (*Chaeropus ecaudatus*) sister to other bandicoots (Carillo et al., 2004; Westerman et al., 2012). Our studies also support a proposed early divergence of the greater bilby, *Macrotis lagotis* from other bandicoots (Carillo et al., 2004; Meredith et al., 2008; Westerman et al., 2010). The greater bilby is known to be drastically different in their genetic make-up, in that it possesses sixteen autosomes and a multiple sex-chromosome system (Westerman et al., 2010), contrasting with the typical bandicoot chromosome set of 2n=14 (Westerman et al., 2012). In addition, our results support the close phylogenetic relationships between the two New Guinean subfamilies Echymiperinae and Peroryctinae (Meredith et al., 2008; Westerman et al., 2012).

~~Order~~ Diprotodontia: Like previous studies our analysis strongly supported the monophyly of Diprotodontia. With the exception of Phalangeriformes the monophyly of the suborders Vombatiformes and Macropodiformes was supported contrasting with one molecular study (Meredith et al., 2009). Amrine-Madsen et al., (2003) found ambivalent support for the monophyly of Phalangeriformes and other studies have been inconclusive (Springer & Kirsch, 1991; Kirsch et al., 1997; Burk et al., 1999). The basal position of Vombatiformes (Vombatidae +

Phascolarctidae) is in agreement with previous molecular studies (Meredith et al., 2009; Nilsson et al., 2010).

The monophyly of the superfamily Phalangeroidea and families Phalangeridae and Burramyidae was supported and in accordance with previous studies (Osborne et al., 2002; Beck, 2008; Phillips & Pratt, 2008; Meredith et al., 2009). Our results are also congruent with previous findings supporting the clade Petauroidea where the families Petauridae and Pseudocheiridae grouped together to the exclusion of Tarsipedidae and Acrobatidae (Osborne et al., 2002; Kavanagh et al., 2004; Phillips & Pratt, 2008; Meredith et al.,; 2009, 2010). Within the family Petuaridae our results contrast recent molecular studies and previous morphological studies, in that the genus *Gymnobelideus* grouped with to the genus *Dactylopsila* to the exclusion of *Petaurus* (Smith, 1984; Aplin & Archer, 1987; Springer et al., 1994; Meredith et al., 2010). These results are more in accordance with the molecular analysis of Edwards & Westerman (1995). Like a previous study (Meredith et al., 2010) we find strong support for the monophyly of the family Pseudocheiridae and three subfamilies. Our results agree with Meredith et al., (2010) in that the genus *Pseudochirops* is paraphyletic due to the placement of the Australian rock-haunting ringtail possum *Petropseudes dahli* with New Guinean species of *Pseudochirops.* Our results are also in agreement with Meredith et al., (2010) in the genera placed in the clades corresponding to the Pseudocheirinae and Pseudochiropsinae in contrast to the assignments by Groves (2005).

Petauroidea grouped with Macropodiformes to the exclusion of Phalangeroidea, contrasting previous studies results (Phillips & Pratt, 2008; Meredith et al., 2009). As shown by previous studies, the three kangaroo families are monophyletic (Osborne et al., 2002; Meredith et al., 2009; Phillips et al., 2013). One interesting result was the consistent placement of the

Sulawesi cuscus, *Strigocuscus celebensis* sister species to the Sulawesi bear, *Ailurops ursinus* rendering the genus *Strigocuscus* paraphyletic. This result agrees with previous molecular studies (Meredith et al., 2009). Within Macropodiformes, the family Hypsiprymnodontidae was sister to a clade consisting of Macropodidae and Potoroidae. This relationship agrees with a previous study (Burk et al., 1998). Within Potoroidae the relationship between *Bettongia* and *Aepyprymnus* to the exclusion of *Potorous* was strongly supported similarly to previous support (Buck et al., 1998). The monophyly of the genus *Petrogale* was strongly supported by this analysis but phylogenetic relationships among species contrast those proposed by Campeau-Peloquin et al., (2001).

Conclusions

Here, we offer a summary phylogeny for marsupials including 80% of the known marsupial diversity, utilizing a Genbank data-mining approach. Overall results are consistent with previous phylogenetic studies and generally recover undisputed deeper level clades suggesting the present phylogenetic hypotheses should serve as valuable tools for future taxonomic and comparative evolutionary studies.

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References

Abello MA. 2013. Analysis of dental homologies and phylogeny of Paucituberculata (Mammalia: Marsupialia). *Biological Journal of the Linnean Society* 109:441-465.

Agnarsson I, May-Collado LJ. 2008. The phylogeny of Cetartiodactyla: the importance of dense taxon sampling, missing data, and the remarkable promise of Cytochrome b to provide reliable species-level phylogenies. *Molecular Evolution and Phylogenetics* 48:964-985.

Amrine-Madsen H, Scally M, Westerman M, Stanhope MJ, Krajewski C, Springer MS. 2003. Nuclear gene sequences provide evidence for the monophyly of australidelphian marsupials *Molecular Phylogenetics and Evolution* 28:186-196.

Aplin KP, Archer M. 1987. Recent advances in marsupial systematics with a new syncretic classification. In: Archer M, ed. *Possums and Opossums: Studies in Evolution.* Surrey Beatty and the Royal Zoological Society of New South Wales: Sydney, 15-72.

Archer M. 1975. *Ningaui*, a new genus of tiny dasyurids (Marsupialia) and two new species, from arid Western Australia, *N. timealeyi* and *N. ridei*. *Memoirs of the Queensland Museum* 17: 237-49.

Archer MA. 1984. The Australian marsupial radiation. In: Archer M, Clayton G, eds. *Vertebrate Zoogeography and Evolution in Australasia*. Hesperian Press: Perth, 633–808.

Archer M, Beck R, Gott M, Hand S, Godhelp H, Black K. 2011. Australia's first fossil marsupial mole (Notoryctemorphia) resolves controversies about their evolution and palaenvironemental origins. *Proceedings of the Royal Society B-Biological Sciences* 278:1498-1506.

Asher RJ, Horovitz I, Sánchez-Villagra MR. 2004. First combined cladistics analysis of marsupial mammal interrelationships. *Molecular Phylogenetics and Evolution* 33:240-250.

Asher M, Kirsch J. 2006. The evolution and classification of marsupials. Armati PJ, Dickman CR, Hume ID. *Marsupials* Cambridge University Press: Cambridge.

Baker ML, Wares JP, Harrison GA, Miller RD. 2004. Relationships among the Families and Orders of Marsupials and the major mammalian lineages based on recombination activating Gene-1. *Journal of Mammalian Evolution* 1:1-16.

Baverstock, PR, Kri M, Birrell, J. 1990. Evolutionary relationships of Australian marsupials as assessed by albumin immunology. *Australian Journal of Zoology* 37:273-287.

Baverstock PR, Kri M, Birrell J, Mckay GM.1990. Albumin immunologic relationships of Australian Marsupials II: The Pseudocheiridae. *Australian Journal of Zoology* 38:519–526.

Beck, MDR. 2008. A dated phylogeny of marsupials using a molecular supermatrix and multiple fossil constraints. *Journal of Mammalogy* 89:175-189.

Blacket MJ, Krajewski C, Labrinidis A, Cambron B, Cooper S, Westerman M. 1999. Systematic relationships within the Dasyurid marsupial tribe Sminthopsini-a multigene approach. *Molecular Phylogenetics and Evolution* 12:140-155.

Bollback JP. 2006. SIMMAP: stochastic character mapping of discrete traits on Phylogenies. *BMC Bioinformatics* 7:88.

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* 47: 457-474.

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

Cardillo M, Bininda-Emonds ORP, Boakes E, Purvis A. 2004. A species-level phylogenetic supertree of marsupials. *Journal of Zoology (London)* 264:11-31.

Campeau-Péloquin A, Kirsch JAW, Eldridge MDB, Lapointe FJ. 2001. Phylogeny of the rock-wallabies, Petrogale (Marsupialia: Macropodidae) based on DNA/DNA hybridization. *Australian Journal of Zoology* 49:463-486.

Case, JA. 1984. A new genus of Potoroinae (Marsupialia: Macropodidae) from the Miocene Ngapalakdi Local Fauna, South Australia, and a definition of the Potoroinae. *Journal of Paleontol* 58:1074–1086.

Colgan, DJ. 1999. Phylogenetic studies of marsupials based on phosphoglycerate kinase DNA sequences. *Molecular Phylogenetics and Evolution* 11:13-26.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. Jmodeltest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.

Edwards D, Westerman M. 1995. The molecular relationships of possum and glider families as revealed by DNA-DNA hybridizations. *Australian Journal of Zoology* 43: 231-240.

Felsenstein, J. 2004. *Inferring Phylogenies*. Sinauer Associates, Sunderland, Massachusetts, pp 531-533.

Flannery, T. 1987. A new species of phalanger (Phalangeridae:Marsupialia) from Montane Western Papua New Guinea. *Records of the Australian Museum* 39: 183-193.

Flores, DA. 2009. Phylogenetic analyses of postcranial skeletal morphology in didelphid marsupials. *Bulletin of the American Museum of Natural History* 320, 81 pp.

Flores A, Abdala F, Giannini NP. 2013. Post-weaning cranial ontogeny in two bandicoots

(Mammalia, Perarnelomorphia, Peramelidae) and comparison with carnivorous marsupials. *Zoology*. 116:372-384.

Frankham G, Handasyde KA, Eldridge MDB. 2012. Novel insights into the phylogenetic relationships of the endangered marsupial genus *Potorus. Molecular Phylogenetics and Evolution* 64: 592-602.

Gallardo MH, Patterson, BD. 1987. An additional 14-chromosome karyotype and sex-chromosome mosaicism in South American marsupials. *Fieldiana Zoology* 39: 111-116.

Gardner, AL. 2005a. Order Didelphimorpha. In Wilson DE, Reeder DM, eds. *Mammal Species of the World: A Taxonomic and Geographic Reference.* 3rd Edition. Baltimore: Johns Hopkins University Press, 3-18.

Gardner, AL. 2005b. Order Paucituberculata, In Wilson DE, Reeder DM, eds. *Mammal Species of the World: A Taxonomic and Geographic Reference.* 3rd Edition. Baltimore: Johns Hopkins University Press, 19-20.

Gardner AL, Creighton GK. 1989. A new generic name for Tate's *microtarsus* group of South American mouse opossums (Marsupialia: Didelphidae). *Proceedings of the Biological Society of Washington* 102: 3-7.

Groves CP. 2005. Order Diprotodontia, In Wilson DE, Reeder DM, eds. *Mammal Species of the*

World: A Taxonomic and Geographic Reference. 3rd Edition. Baltimore: Johns Hopkins University Press, 43-70.

Harvey PH, Pagel A. 1991. Comparative methods for explaining adaptations. *Nature* 351:619-654.

Hayman DL, Martin PG. 1974. Monetramata and Marsupialia. In: B. John, ed. *Animal Cytogenetics*. Berlin*:* Berlin Gebruder Borntraer.

Horovitz I, Sánchez-Villagra MR. 2003. A morphological analysis of marsupial mammal higher-level phylogenetic relationships. *Cladistics* 19:181-212.

Huelsenbeck JP, Ronquist F. 2001. mrbayes: Bayesian inference of phylogenetic trees *Bioinformatics*. 17:754-755.

Hughes, RL. 1965. Comparative morphology of spermatozoa from five marsupial families. *Australian Journal of Zoology*. 13:533-543.

Isaac NJB, Turvey ST, Collen B, Waterman C, Baillie JEM. 2007. Mammals on the EDGE: conservation priorities based on threat and phylogeny. *PloS ONE* 2(3): e296.

Jansa SA, Forsman JF, Voss RS. 2006. Different patterns of selection on the nuclear genes IRBP and DMP-1 affect the efficiency but not the outcome of phylogeny estimation for didelphid marsupials. *Molecular Phylogenetics and Evolution* 38: 363-380.

Kavanagh JR, 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.

Kirsch JAW. 1977. *The comparative serology of Marsupialia, and a classification of marsupials.* Australian Journal of Zoology Supplementary Series No. 52, 152 pp.

Kirsch JAW, Calaby JH. 1977. The species of living marsupials: an annotated list. In Stonehouse B, Gilmore DP, eds. *The biology of marsupials*. London: Macmillian, 9-26.

Kirsch JAW, Dickerman AW, Reig OA, Springer MS. 1991. DNA hybridization evidence for the Australasian affinity of the American marsupial *Dromiciops australis*. *Proceeding of the National Academy of Sciences of the USA* 88: 10465-10469.

Kirsch JAW, Lapointe FJ, Springer MS. 1997. DNA hybridization studies of marsupials and their implications for Metatherien classification. *Australian Journal of Zoology* 45:211-280.

Krajewski C, Blacket M, Buckley L, Westerman M. 1997. A multigene assessment of phylogenetic relationships within the Dasyurid marsupial subfamily Sminthopsinae. *Molecular Phylogenetics and Evolution* 8: 236-248.

Krajewski C, Anderson FE, Woolley PA, Westerman M. 2012. Molecular evidence for a deep clade of dunnarts (Marsupialia: Dasyuridae: *Sminthopsis*). *Journal of Mammalian Evolution* 19: 265-276.

Kullander K, Carlson B, Hallbook F. 1997. Molecular phylogeny and evolution of the neurotrophins of monotremes and marsupials. *Journal of Molecular Evolution* 45: 311–321

Ladèveze S, de Muizon C. 2010. Evidence of early evolution of Australidephia (Metatheria, Mammalia) in South America : phylogenetic relationships of the metatherians from the Late Palaeocene of Itaborai (Brazil) based on teeth and petrosal bones. *Zoological Journal of the Linnean Society* 159:746-784

Maddison W P, Maddison DR. 2011. Mesquite: a modular system for Evolutionary analysis Version 2.75 http://mesquiteprojectorg.

Malekian M, Cooper SJB, Norman JA, Christidis L, Carthew SM. 2010. Molecular systematics and evolutionary origins of the genus *Petaurus* (Marsupialia: Petauridae) in Australia and New Guinea. *Molecular Phylogenetics and Evolution* 54:122-135.

Marshall, L G. 1980. Systematics of the South American marsupial family Caenolestidae. *Fieldiana Geology* 5:1-145.

Marshall LG, Case JA, Woodburne MO. 1990. Phylogenetic relationships of the families of marsupials. In HH Genoways, ed. *Current Mammalogy*. New York: Plenum Press, 433-506.

May-Collado LJ, Agnarsson I. 2006. Cytochrome b and Bayesian inference of whale phylogeny. *Molecular Phylogenetics and Evolution* 38: 344–354.

Mckay GM.1984. Cytogenetic relationships of possums and gliders. In AP Smith, ID Hume, eds. *Possums and gliders*. Sydney, Australia: Beatty and Sons with Australian Mammal Society, 9-16

Mckenna MC, Bell SK. 1997. *Classification of mammals above the species level*. Columbia University Press, New York.

Meredith RW, Westerman M, Springer MS. 2008a. A timescale and phylogeny for "Bandicoots" (Peramelemorphia: Marsupialia) based on sequences for five nuclear genes. *Molecular Phylogenetics and Evolution* 47:1-20.

Meredith RW, Westerman M, Springer MS. 2008b. A phylogeny and timescale for the living genera of kangaroos and kin (Macropodiformes: Marsupialia) based on nuclear DNA sequences. *Australian Journal of Zoology* 56:395-410.

Meredith RW, Westerman M, Springer MS. 2009. A phylogeny of Diprotodontia (Marsupialia) based on sequences for five nuclear genes. *Molecular Phylogenetics and Evolution* 51:554-571.

Meredith RW, Mendoza MA, Roberts KK, Westerman M, Springer MS. 2010. A phylogeny and timescale for the evolution of Pseudocheiridae (Marsupialia: Diprotodontia) in Australia and New Guinea. *Journal of Mammalian Evolution* 17: 75-99.

Munemasa M, Nikaido M, Nishihara H, Donnellan S, Austin CC, Okada N. 2008. Newly discovered young CORE-sines in Marsupial genomes. *Gene* 407: 176-185

Nilsson MA, Gullberg A, Spotorno AE, Arnason U, Janke A. 2003. Radiation of extant marsupials after the K/T boundary: evidence from complete mitochondrial genomes. *Journal of Molecular Evolution* 57: S3-S12

Nilsson MA, Arnason U, Spencer PBS, Janke A. 2004. Marsupial relationships and a timeline for marsupial radiation in South Gondwana. *Gene* 340, 189-196.

Nilsson MA, Churakov G, Sommer M, Van Tran N, Zemann A, Brosius J, Schmitz J. 2010. Tracking marsupial evolution using archaic genomic retroposon insertions. *PloS Biol* 8: e1000436.

Osborne M J, Christidis L, Norman JA. 2002. Molecular phylogenetics of the Diprotodontia (kangaroos, wombats, koala, possums, and allies). *Molecular Phylogenetics and Evolution* 25: 219-228.

Palma RE, Spotorno AE. 1999. Molecular systematics of marsupials based on the rrna 12S mitochondrial gene: the phylogeny of Didelphimorphia and of the living fossil Microbiotheriid

Dromiciops gliroides Thomas. *Molecular Phylogenetics and Evolution* 13:525-535

Phillips MJ, Mclenachan PA, Down C, Gibb GC, Penny D. 2006. Combined mitochondrial and nuclear DNA sequences resolve the interrelates of the major Australasian Marsupial radiations. *Systematic Biology* 55:122-137.

Phillips MJ, Pratt RC. 2008. Family-level relationships among the Australasian marsupial "herbivores" (Diprotodontia: Koala, wombats, kangaroos and possums). *Molecular Phylogenetics and Evolution* 46: 594-605.

Phillips M, Haouchar D, Pratt RC, Gibb GC, Bunce M. 2013. Inferring kangaroo phylogeny from incongruent nuclear and mitochondrial genes. *PloS One* 8: e57745.

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and Bayesian approaches over likelihood ratio tests *Systematic Biology* 53:793-808.

Prideaux GJ, Warburton N. 2010. An osteology-based appraisal of the phylogeny and evolution of kangaroos and wallabies (Macropodidae: Marsupialia). *Zoological Journal of the Linnean Society* 159: 954-987.

Raterman D, Meredith RW, Ruedas LA, Springer MS. 2006. Phylogenetic relationships of the cuscuses and brushtail possums (Marsupialia: Phalangeridae) using the nuclear gene BRCA1.

Australian Journal of Zoology 54: 353-361.

Retief JD, Krajewski C, Westerman M, Winkfein RJ, Dixon GH. 1995. Molecular phylogeny and evolution of marsupial Protamine P1 genes. *Proceedings of the Royal Society London B* 259: 7-14.

Ride WDL.1961. The cheek-teeth of Hypsiprymnodon moschatus Ramsay 1876 (Macropodidae: Marsupialia). *Journal and Proceedings of the Royal Society of Western Australia* 44: 53–60.

Ronquist F, Huelsenbeck JP. 2003. Mrbayes 3: Bayesian Phylogenetic Inference under mixed models. *Bioinformatics* 19, 1572-1574.

Sánchez-Villagra MR. 2001. The phylogenetic relationships of argyrolagid marsupials. *Zoological Journal of the Linnean Society* 131:481-496

Sánchez-Villagra MR, Goswami A, Weisbecker V, Mock O, Kuratani S. 2008. Conserved relative timing of cranial ossification patterns in early mammalian evolution. *Evolution and Development* 10: 519-530

Sanclair EA, Westerman M. 1997. Phylogenetic relationships within the genus *Potorus* (Marsupialia: Potoroidea) based on allozyme electrophoresis and sequence analysis of the cytochrome b gene. *Journal of Mammalian Evolution* 4:147-161

Shimodaira H, Hasawa M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* 16, 1114-1116

Smith A. 1984. The species and possums and gliders. In: A Smith, I Hume eds. *Possums and Gliders*. Sydney, Australia: Beatty and Sons with Australian Mammal Society, 13-15.

Springer MS. 1993. Phylogeny and rates of character evolution among ringtail possums (Pseudocheridae, Marsupialia). *Australia Journal of Zoology* 41:273-291.

Springer MS, Woodburke MO. 1989. The distribution of some basicranial characters within the Marsupialia and a phylogeny of the Phalangeriformes. *Journal of Vertebrate Paleontology* 9:210-221.

Springer MS, Westerman M, Kirsch JAW. 1994. Relationships among orders and Families of marsupials based on 12S ribosomal DNA sequences and the timing of The marsupial radiation. *Journal of Mammalian Evolution* 2:85–115.

Springer MS, Kirsch JAW. 1989. Rates of single-copy DNA evolution in phalangeriform marsupials. *Molecular Biology and Evolution* 6:331-341.

Springer MS, Kirsch JAW.1991. DNA hybridization, the compression effect, and the radiation of diprotodontian marsupials. *Systematic Zoology* 40:131-151.

Springer MS, Kirsch JAW, Case JA. 1997. The chronicle of marsupial evolution In: T Givinish , K Sytsma, eds. *Molecular Evolution and Adaptive Radiations*. New York: Cambridge University Press, 129–161.

Springer MS, Westerman M, Kavanagh JR, Burk A, Woodburne MO, Kao DJ, Krajewski C. 1998. The origin of the Australasian marsupial fauna and the phylogenetic affinities of the enigmatic monitor del monte and marsupial mole. *Proceedings of the Royal Society London B* 265: 2381-2386

Swofford, DL. 2003. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4. Sinauer Associates, Sunderland, Massachusetts

Szalay FS. 1982. Phylogenetic relationships of the marsupials. *Geobios Mémoire Spécial* 6:177–190.

Voss RS, Jansa SA. 2009. Phylogenetic relationships and classification of didelphid marsupials an extant radiation of the new world metatherian mammals. *Bulletin of the American Museum of Natural History* 322:1-117.

Voss RS, Lunde DP, Jansa SA.2005. On the contents of *Gracilinanus* Gardner and Creighton, 1989, with the description of a previously unrecognized clade of small didelphid marsupials. *American Museum Novitates* 3482:1-34.

Weisbecker V, Goswami A, Wroe S, Sánchez-Villagra MR. 2008. Ossification heterochrony in the therian postcranial skeleton and the marsupial-placental dichotomy. *Evolution* 62: 2027-2041.

Westerman M, Woolley PA. 1990. Cytogenetics of some New Guinean dasyurids and genemo evolution in the Dasyuridae (Marsupialia). *Australian Journal of Zoology* 37:521-31.

Westerman M, Janczewski DN, O'Brien SJ. 1990. DNA–DNA hybridization studies and marsupial phylogeny. *Australian Journal of Zoology* 37: 315–323.

Westerman M, Meredith RW, Springer MS. 2010. Cytogenetics meets phylogenetics: a review of karyotype evolution in diprotodontian marsupials. *Journal of Heredity* 101:690-702.

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

Wilson DE, Reeder DM. 2005. *Mammal Species of the World: A Taxonomic and Geographic Reference*. 3rd edition. Baltimore: Johns Hopkins University Press.

Wroe S, Ebatch M, Ahyong S, de Muizon C, Muirhead J. 2000. Cladistic analysis of dasyuromorphian (Marsupialia): Phylogeny using cranial and dental characters. *Journal of*

Mammalogy 81:1008-1024.

Table 1. The Shimodaira-Hasagawa test did not reject alternative basal topologies when compared to the preferred tree (concatenated analysis removing taxa with <10% character data cover).

Figure Legends

Figure 1. Summary cladogram of all the analyses showing support for relationships among major clades within Metatheria. Photos obtained from Wikimedia Commons.

Figure 2. Majority rule consensus of the Bayesian analyses using the full concatenated character matrix (excluding species with less than 10% data coverage) for Ameridelphia. Note that *Micoureus* was recently recognized as a subgenus of *Marmosa* (IUCN Red List of Threatened Spcies 2013.2).

Figure 3. Majority rule consensus of the Bayesian analyses using the full concatenated character matrix (excluding species with less than 10% data coverage) for Australasian marsupials: Notoryctemorphia, Peramelemorphia, and Dasyuromorphia. Note that the species *Antechinus naso, Antechinus melanurus,* and *Paramurexia rothschildi* are now recognized as members of the genus *Murexia* (IUCN Red List of Threatened Spcies 2013.2).

Figure 4. Majority rule consensus of the Bayesian analyses using the full concatenated character matrix (excluding species with less than 10% data coverage) for Australasian marsupials: Diprotodontia.

Supplementary Material

Table S1. Species included in each analysis and their respective GenBank Accession number. Note that *Micoureus* was recently recognized as a subgenus of *Marmosa,* and *Antechinus naso, Antechinus melanurus,* and *Paramurexia rothschildi* are now recognized as members of the genus *Murexia* (IUCN Red List of Threatened Spcies 2013.2).

Figure S1. Metatheria majority rule consensus of the Bayesian analyses using the full concatenated character matrix. Note that *Micoureus* was recently recognized as a subgenus of *Marmosa,* and *Antechinus naso, Antechinus melanurus,* and *Paramurexia rothschildi* are now recognized as members of the genus *Murexia* (IUCN Red List of Threatened Spcies 2013.2).

Figure S2. Metatheria majority rule consensus of the Bayesian analyses using the full concatenated character matrix excluding protamine. Note that *Micoureus* was recently recognized as a subgenus of *Marmosa,* and *Antechinus naso, Antechinus melanurus,* and *Paramurexia rothschildi* are now recognized as members of the genus *Murexia* (IUCN Red List of Threatened Spcies 2013.2)

Figure S3. Metatheria majority rule consensus of the Bayesian analyses using the nuDNA concatenated character matrix excluding protamine. Note that *Micoureus* was recently recognized as a subgenus of *Marmosa,* and *Antechinus naso, Antechinus melanurus,* and *Paramurexia rothschildi* are now recognized as members of the genus *Murexia* (IUCN Red List of Threatened Spcies 2013.2)

Figure S4. Metatheria majority rule consensus of the Bayesian analyses using the mtDNA concatenated character matrix. Note that *Micoureus* was recently recognized as a subgenus of

Marmosa, and *Antechinus naso, Antechinus melanurus,* and *Paramurexia rothschildi* are now recognized as members of the genus *Murexia* (IUCN Red List of Threatened Spcies 2013.2).

Figure 1(on next page)

Summary cladogram of all the analyses showing support for relationships among major clades within Metatheria Photos obtained from Wikimedia Commons

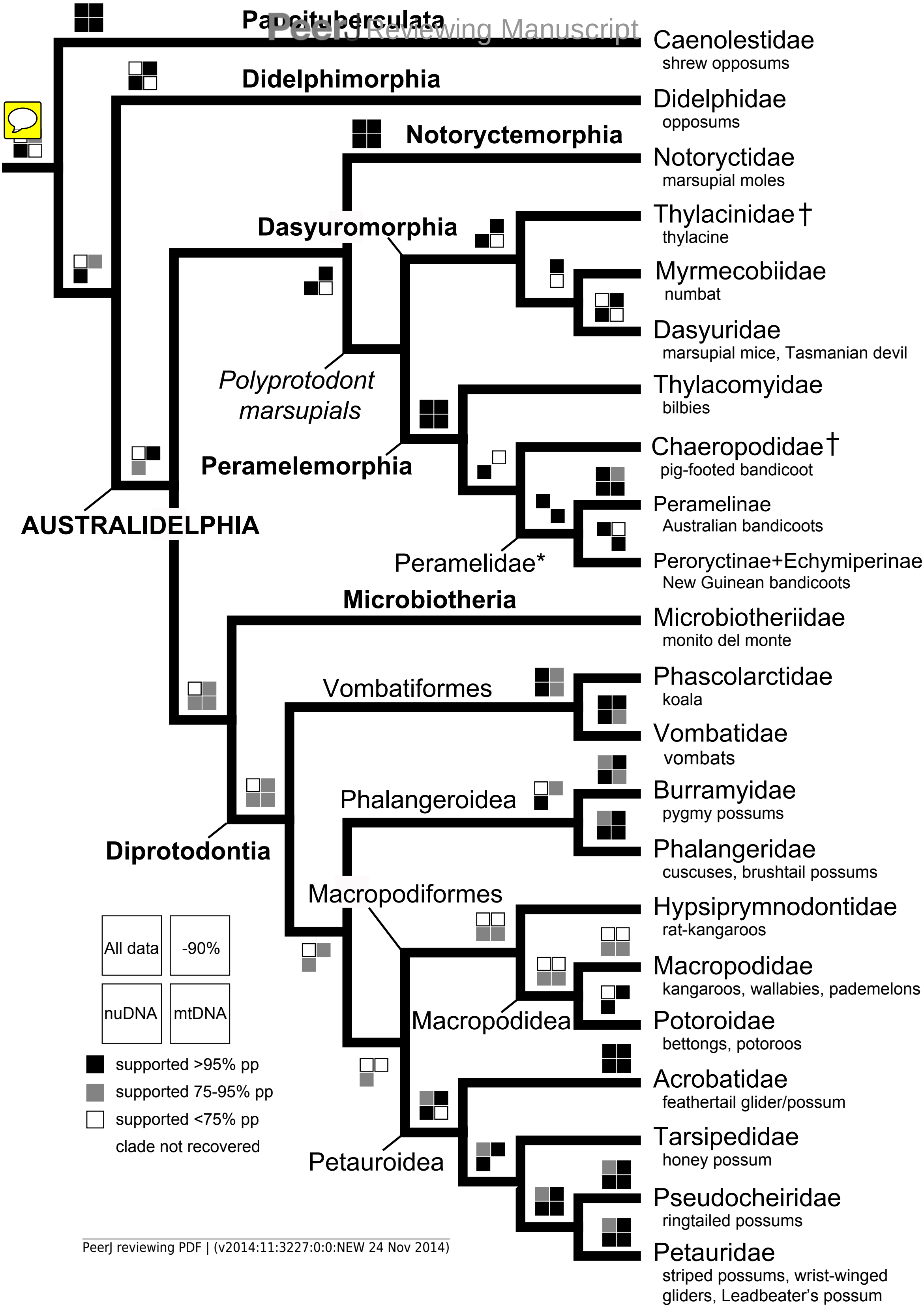
Paucituberculata
Caenolestidae
shrew opposums
Didelphimorphia
Didelphidae
opposums
Notoryctemorphia
Notoryctidae
marsupial moles
Dasyuromorphia
Thylacinidae†
thylacine
Myrmecobiidae
numbat
Dasyuridae
marsupial mice, Tasmanian devil
Polyprotodont marsupials
Peramelemorphia
Thylacomyidae
bilbies
Chaeropodidae†
pig-footed bandicoot
Peramelinae
Australian bandicoots
Peramelidae*
Peroryctinae+Echymiperinae
New Guinean bandicoots
AUSTRALIDELPHIA
Microbiotheria
Microbiotheriidae
monito del monte
Vombatiformes
Phascolarctidae
koala
Vombatidae
vombats
Phalangeroidea
Burramyidae
pygmy possums
Phalangeridae
cuscuses, brushtail possums
Diprotodontia
Macropodiformes
Hypsiprymnodontidae
rat-kangaroos
Macropodidae
kangaroos, wallabies, pademelons
Macropodidea
Potoroidae
bettongs, potoroos
Acrobatidae
feathertail glider/possum
Petauroidea
Tarsipedidae
honey possum
Pseudocheiridae
ringtailed possums
Petauridae
striped possums, wrist-winged gliders, Leadbeater's possum
All data
-90%
nuDNA
mtDNA
■ supported >95% pp
▨ supported 75-95% pp
☐ supported <75% pp
clade not recovered

Figure 2(on next page)

Figure 2. Majority rule consensus of the Bayesian analyses using the full concatenated character matrix (excluding species with less than 10% data coverage)for Ameridelphia.

Note that *Micoureus* was recently recognized as a subgenus of *Marmosa* (IUCN Red List of Threatened Spcies 2013.2).

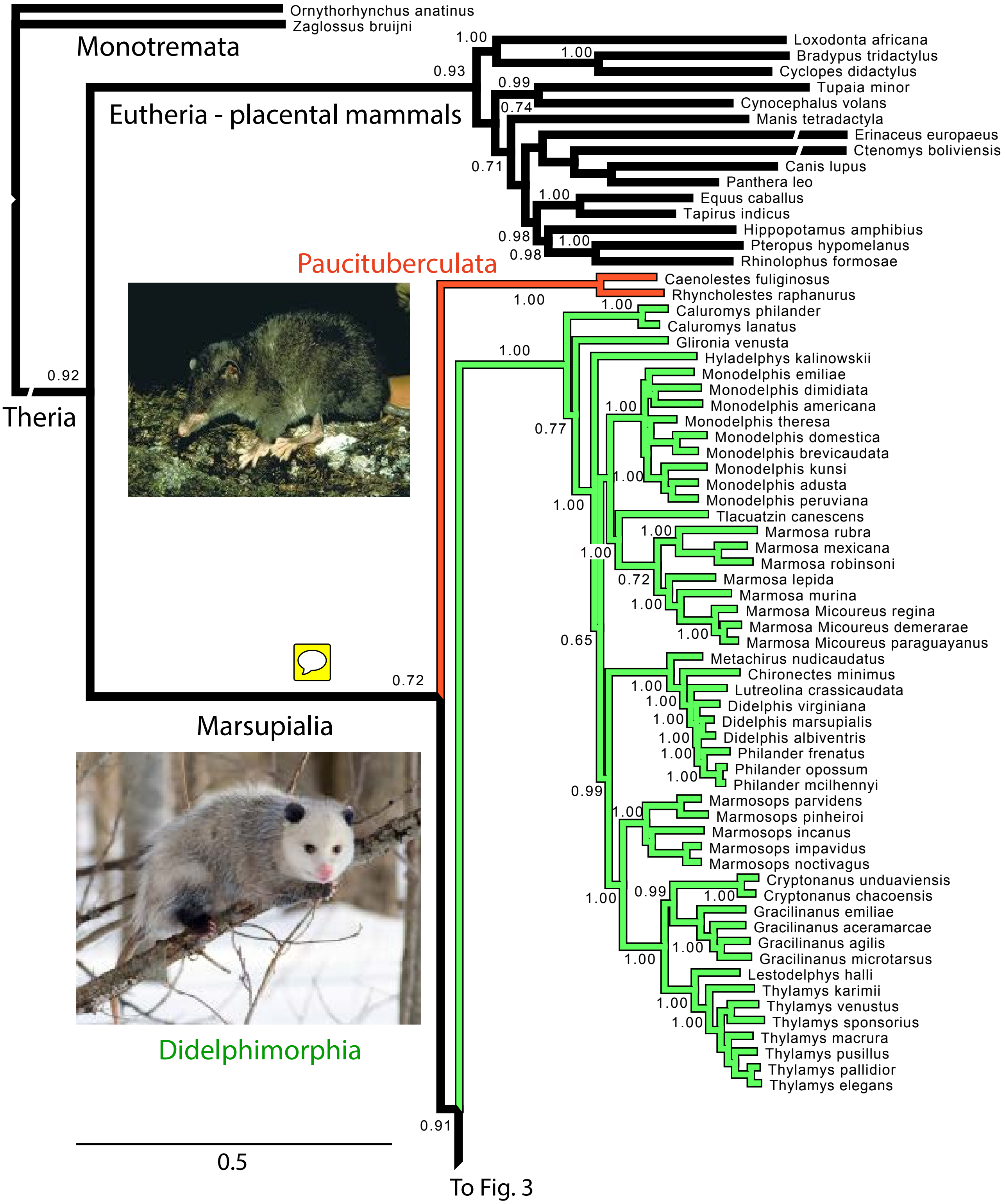
Ornythorhynchus anatinus
Zaglossus bruijni
Monotremata
1.00
Loxodonta africana
1.00
Bradypus tridactylus
0.93
Cyclopes didactylus
0.99
Tupaia minor
0.74
Cynocephalus volans
Eutheria - placental mammals
Manis tetradactyla
Erinaceus europaeus
Ctenomys boliviensis
0.71
Canis lupus
Panthera leo
1.00
Equus caballus
Tapirus indicus
0.98
Hippopotamus amphibius
1.00
Pteropus hypomelanus
0.98
Rhinolophus formosae
Paucituberculata
Caenolestes fuliginosus
Rhyncholestes raphanurus
1.00
1.00
Caluromys philander
Caluromys lanatus
1.00
Glironia venusta
Hyladelphys kalinowskii
Monodelphis emiliae
0.92
Monodelphis dimidiata
1.00
Monodelphis americana
Theria
0.77
Monodelphis theresa
Monodelphis domestica
Monodelphis brevicaudata
Monodelphis kunsi
1.00
Monodelphis adusta
1.00
Monodelphis peruviana
Tlacuatzin canescens
1.00
Marmosa rubra
1.00
Marmosa mexicana
Marmosa robinsoni
0.72
Marmosa lepida
1.00
Marmosa murina
Marmosa Micoureus regina
1.00
Marmosa Micoureus demerarae
0.65
Marmosa Micoureus paraguayanus
Metachirus nudicaudatus
0.72
Chironectes minimus
1.00
Lutreolina crassicaudata
Marsupialia
1.00
Didelphis virginiana
1.00
Didelphis marsupialis
1.00
Didelphis albiventris
1.00
Philander frenatus
Philander opossum
1.00
Philander mcilhennyi
0.99
Marmosops parvidens
1.00
Marmosops pinheiroi
Marmosops incanus
Marmosops impavidus
Marmosops noctivagus
Cryptonanus unduaviensis
0.99
1.00
1.00
Cryptonanus chacoensis
Gracilinanus emiliae
Gracilinanus aceramarcae
1.00
Gracilinanus agilis
1.00
Gracilinanus microtarsus
Lestodelphys halli
Didelphimorphia
Thylamys karimii
1.00
Thylamys venustus
1.00
Thylamys sponsorius
Thylamys macrura
Thylamys pusillus
Thylamys pallidior
Thylamys elegans
0.91
0.5
To Fig. 3

Figure 3(on next page)

Majority rule consensus of the Bayesian analyses using the full concatenated character matrix (excluding species with less than 10% data coverage) for Australasian marsupials.

Notoryctemorphia, Peramelemorphia, and Dasyuromorphia. Note that the species *Antechinus naso, Antechinus melanurus,* and *Paramurexia rothschildi* are now recognized as members of the genus *Murexia* (IUCN Red List of Threatened Spcies 2013.2).

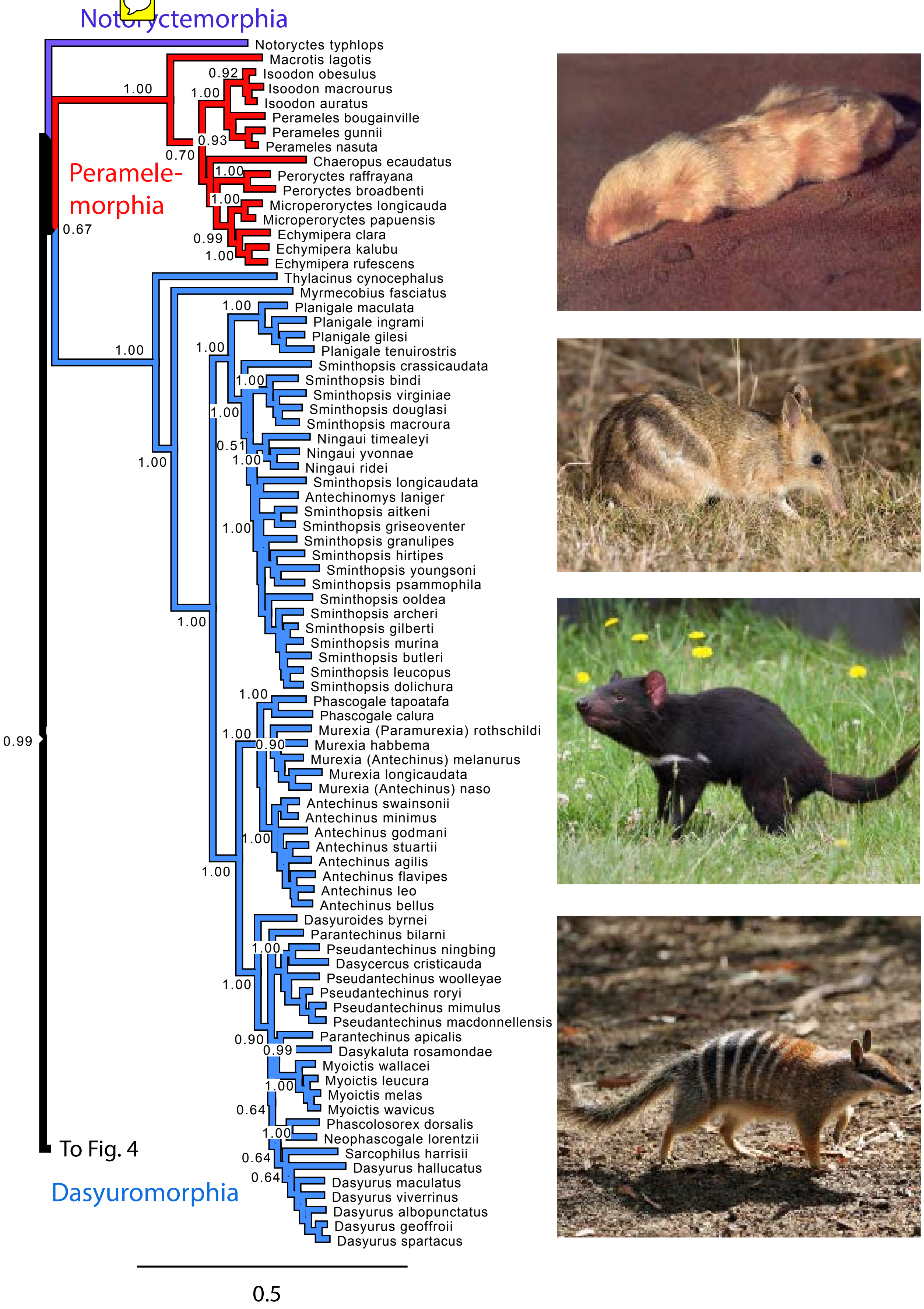
Notoryctemorphia
Notoryctes typhlops
Macrotis lagotis
0.92
Isoodon obesulus
1.00
1.00
Isoodon macrourus
Isoodon auratus
Perameles bougainville
Perameles gunnii
0.93
Perameles nasuta
0.70
Chaeropus ecaudatus
1.00
Peroryctes raffrayana
Peramele-
morphia
Peroryctes broadbenti
1.00
Microperoryctes longicauda
Microperoryctes papuensis
0.67
0.99
Echymipera clara
Echymipera kalubu
1.00
Echymipera rufescens
Thylacinus cynocephalus
Myrmecobius fasciatus
1.00
Planigale maculata
Planigale ingrami
Planigale gilesi
1.00
1.00
Planigale tenuirostris
Sminthopsis crassicaudata
1.00
Sminthopsis bindi
Sminthopsis virginiae
1.00
Sminthopsis douglasi
Sminthopsis macroura
Ningaui timealeyi
0.51
Ningaui yvonnae
1.00
1.00
Ningaui ridei
Sminthopsis longicaudata
Antechinomys laniger
Sminthopsis aitkeni
1.00
Sminthopsis griseoventer
Sminthopsis granulipes
Sminthopsis hirtipes
Sminthopsis youngsoni
Sminthopsis psammophila
Sminthopsis ooldea
Sminthopsis archeri
1.00
Sminthopsis gilberti
Sminthopsis murina
Sminthopsis butleri
Sminthopsis leucopus
Sminthopsis dolichura
1.00
Phascogale tapoatafa
Phascogale calura
0.99
Murexia (Paramurexia) rothschildi
1.00
0.90
Murexia habbema
Murexia (Antechinus) melanurus
Murexia longicaudata
Murexia (Antechinus) naso
Antechinus swainsonii
Antechinus minimus
Antechinus godmani
1.00
Antechinus stuartii
Antechinus agilis
1.00
Antechinus flavipes
Antechinus leo
Antechinus bellus
Dasyuroides byrnei
Parantechinus bilarni
1.00
Pseudantechinus ningbing
Dasycercus cristicauda
Pseudantechinus woolleyae
1.00
Pseudantechinus roryi
Pseudantechinus mimulus
Pseudantechinus macdonnellensis
0.90
Parantechinus apicalis
0.99
Dasykaluta rosamondae
Myoictis wallacei
Myoictis leucura
1.00
Myoictis melas
0.64
Myoictis wavicus
Phascolosorex dorsalis
1.00
Neophascogale lorentzii
To Fig. 4
0.64
Sarcophilus harrisii
Dasyurus hallucatus
0.64
Dasyuromorphia
Dasyurus maculatus
Dasyurus viverrinus
Dasyurus albopunctatus
Dasyurus geoffroii
Dasyurus spartacus
0.5

Figure 4(on next page)

Majority rule consensus of the Bayesian analyses using the full concatenated character matrix (excluding species with less than 10% data coverage) for Australasian marsupials: Diprotodontia.

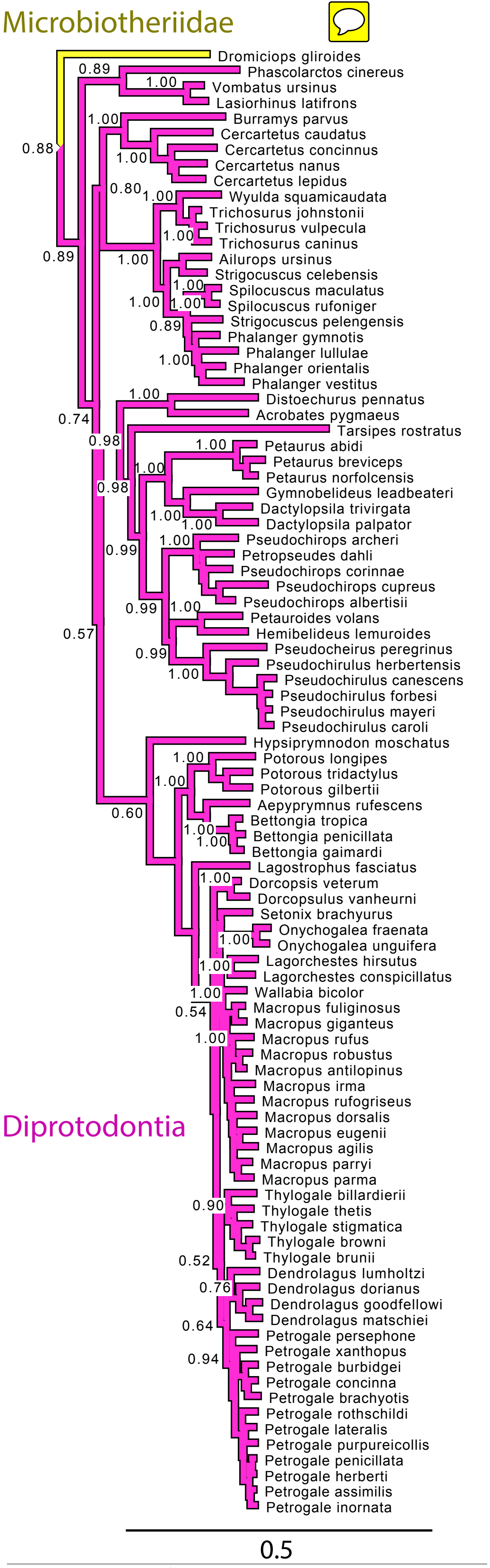
Microbiotheriidae
Dromiciops gliroides
0.89
Phascolarctos cinereus
1.00
Vombatus ursinus
Lasiorhinus latifrons
1.00
Burramys parvus
Cercartetus caudatus
0.88
1.00
Cercartetus concinnus
Cercartetus nanus
Cercartetus lepidus
0.80
1.00
Wyulda squamicaudata
Trichosurus johnstonii
Trichosurus vulpecula
1.00
Trichosurus caninus
0.89
1.00
Ailurops ursinus
Strigocuscus celebensis
1.00
Spilocuscus maculatus
1.00
1.00
Spilocuscus rufoniger
0.89
Strigocuscus pelengensis
Phalanger gymnotis
1.00
Phalanger lullulae
Phalanger orientalis
Phalanger vestitus
1.00
Distoechurus pennatus
0.74
Acrobates pygmaeus
Tarsipes rostratus
0.98
1.00
Petaurus abidi
Petaurus breviceps
1.00
Petaurus norfolcensis
0.98
Gymnobelideus leadbeateri
Dactylopsila trivirgata
1.00
1.00
Dactylopsila palpator
1.00
Pseudochirops archeri
0.99
Petropseudes dahli
Pseudochirops corinnae
Pseudochirops cupreus
Pseudochirops albertisii
0.99
1.00
Petauroides volans
0.57
Hemibelideus lemuroides
0.99
Pseudocheirus peregrinus
Pseudochirulus herbertensis
1.00
Pseudochirulus canescens
Pseudochirulus forbesi
Pseudochirulus mayeri
Pseudochirulus caroli
Hypsiprymnodon moschatus
1.00
Potorous longipes
Potorous tridactylus
1.00
Potorous gilbertii
Aepyprymnus rufescens
0.60
Bettongia tropica
1.00
Bettongia penicillata
1.00
Bettongia gaimardi
Lagostrophus fasciatus
1.00
Dorcopsis veterum
Dorcopsulus vanheurni
Setonix brachyurus
Onychogalea fraenata
1.00
Onychogalea unguifera
Lagorchestes hirsutus
1.00
Lagorchestes conspicillatus
1.00
Wallabia bicolor
Macropus fuliginosus
0.54
Macropus giganteus
1.00
Macropus rufus
Macropus robustus
Macropus antilopinus
Macropus irma
Macropus rufogriseus
Diprotodontia
Macropus dorsalis
Macropus eugenii
Macropus agilis
Macropus parryi
Macropus parma
Thylogale billardierii
0.90
Thylogale thetis
Thylogale stigmatica
Thylogale browni
Thylogale brunii
0.52
Dendrolagus lumholtzi
0.76
Dendrolagus dorianus
Dendrolagus goodfellowi
Dendrolagus matschiei
0.64
Petrogale persephone
Petrogale xanthopus
0.94
Petrogale burbidgei
Petrogale concinna
Petrogale brachyotis
Petrogale rothschildi
Petrogale lateralis
Petrogale purpureicollis
Petrogale penicillata
Petrogale herberti
Petrogale assimilis
Petrogale inornata
0.5

Table 1(on next page)

The Shimodaira-Hasagawa test did not reject alternative basal topologies when compared to the preferred tree (concatenated analysis removing taxa with <10% character data cover).

Table 1. The Shimodaira-Hasagawa test did not reject alternative basal topologies when compared to the preferred tree (concatenated analysis removing taxa with <10% character data cover).

Tree	-lnL	Diff-ln L	P
Ameridelphia monophyletic	435351.2	5.84062	0.463
Didelphimorphia is sister to the remaining Marsupialia	435353.4	2.22915	0.725
Australian Australidelphia is monophyletic (that is Microbiotheriidae is sister to other Australidelphia) (Nilsson et al., 2010)	435361.1	9.91611	0.263