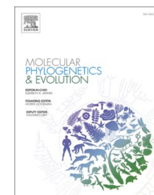
Molecular Phylogenetics and Evolution 158 (2021) 107082

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev



Enhancing mitogenomic phylogeny and resolving the relationships of extinct megafaunal placental mammals

Matthew J. Phillips [a,*], Sarah Shazwani Zakaria [a,b]

[a] School of Biology and Environmental Science, Queensland University of Technology, 2 George Street, Brisbane 4000, QLD, Australia
[b] School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) Caw. Negeri Sembilan, Kuala Pilah 72000, Malaysia

ARTICLE INFO

Keywords:
Ancient DNA
Nucleotide composition
Hydrophobicity
Mitochondria
Placentalia
RY coding

ABSTRACT

Mitochondrial genomes provided the first widely used sequences that were sufficiently informative to resolve relationships among animals across a wide taxonomic domain, from within species to between phyla. However, mitogenome studies supported several anomalous relationships and fell partly out of favour as sequencing multiple, independent nuclear loci proved to be highly effective. A tendency to blame mitochondrial DNA (mtDNA) has overshadowed efforts to understand and ameliorate underlying model misspecification. Here we find that influential assessments of the infidelity of mitogenome phylogenies have often been overstated, but nevertheless, substitution saturation and compositional non-stationarity substantially mislead reconstruction. We show that RY coding the mtDNA, excluding protein-coding 3rd codon sites, partitioning models based on amino acid hydrophobicity and enhanced taxon sampling improve the accuracy of mitogenomic phylogeny reconstruction for placental mammals, almost to the level of multi-gene nuclear datasets. Indeed, combined analysis of mtDNA with 3-fold longer nuclear sequence data either maintained or improved upon the nuclear support for all generally accepted clades, even those that mtDNA alone did not favour, thus indicating "hidden support". Confident mtDNA phylogeny reconstruction is especially important for understanding the evolutionary dynamics of mitochondria themselves, and for merging extinct taxa into the tree of life, with ancient DNA often only accessible as mtDNA. Our ancient mtDNA analyses lend confidence to the relationships of three extinct megafaunal taxa: glyptodonts are nested within armadillos, the South American ungulate, *Macrauchenia* is sister to horses and rhinoceroses, and sabre-toothed and scimitar cats are the monophyletic sister-group of modern cats.

1. Introduction

Resolving relationships among the radiation of placental mammal orders has been a major success for molecular phylogenetics. Mitochondrial (mt) genomes provided the first large (>10 kb) molecular datasets that were sampled broadly enough to address such deep-level relationships between animal orders. They were enthusiastically embraced and gave the first statistically strong support for many now accepted relationships among placental mammals (e.g. Xu et al., 1996; Penny and Hasegawa, 1997; Waddell et al., 1999) and marsupials (e.g. Phillips et al., 2001; Nilsson et al., 2003). However, several anomalous results, such as rodent paraphyly (D'Erchia et al., 1996), grouping monotremes with marsupials (Janke et al., 1997) and studies placing passerines (perching birds) rather than palaeognaths as sister to all other living birds (e.g. Härlid and Arnason, 1999) diminished the reputation of mitogenome phylogeny, even though the accepted tree was not statistically rejected in most cases. At the same time, concatenated nuclear genes were beginning to show promise, such as identifying a diverse African mammal clade (Springer et al., 1997). The relationships of the placental orders have since been largely resolved (Fig. 1) with nuclear gene sequences and rare genomic events (Madsen et al., 2001; Murphy et al., 2001a; Murphy et al., 2001b; Waddell et al., 2001; Kriegs et al., 2006; Nishihara et al., 2006; Prasad et al., 2008; Meredith et al., 2011; Hallström and Janke, 2010; Song et al., 2012; Liu et al., 2017).

The standing of mtDNA for resolving deeper level phylogeny has collapsed further in the face of influential critiques (Springer et al., 2001; Ballard and Whitlock, 2004; Galtier et al., 2009; Morgan et al., 2014; but see Rubinoff and Holland, 2005) that emphasized recalcitrant phylogenetic properties of mtDNA evolution. Mitogenomes do however, have many beneficial phylogenetic properties and to unlock these the emphasis instead needs to be placed on overcoming substitution model misspecification. The highly conserved gene order of mitogenomes

* Corresponding author.
E-mail address: m9.phillips@qut.edu.au (M.J. Phillips).

https://doi.org/10.1016/j.ympev.2021.107082
Received 10 October 2020; Received in revised form 21 December 2020; Accepted 11 January 2021
Available online 20 January 2021
1055-7903/© 2021 Elsevier Inc. All rights reserved.

provides clear homology over hundreds of millions of years (Pereira, 2000), while rapid mutation rates generate strong phylogenetic signals over short time frames, and yet, sites under strong purifying selection have very low substitution rates (Pesole et al., 1999; Havird and Sloan, 2016). Together, these features provide a uniquely wide domain of utility, making mitochondrial sequences attractive as phylogenetic markers – from populations through to phyla. Furthermore, haploidy, maternal inheritance, and non-recombinant linkage (which also allows strong selective sweeps from few positively selected sites) typically lower the effective population size of mtDNA, resulting in shorter coalescence times (Zink and Barrowclough, 2008). This in turn is expected to reduce the influence of incomplete lineage sorting, and thus improve resolution of rapid radiations, with mitogenomes more likely to reflect "true" speciation histories than individual nuclear genes (Funk and Omland, 2003).

The flip side of mitogenomes being single linkage units is that their loci do not provide independent estimates of the species tree (Moore, 1995). Furthermore, the selective sweeps and paucity of recombination that enhance the potential of mitogenomes as speciation indicators also render them susceptible to Muller's ratchet promoting introgression in association with fitness differences across populations (e.g. Ropiquet and Hassanin, 2006; Melo-Ferreira et al., 2012; Phillips et al., 2013). Thus, high throughput sequencing of multiple nuclear loci diminishes the value of shallower mtDNA coalescence, by offering a far more extensive examination of species histories.

The most influential arguments against using mtDNA as phylogenetic markers have been inaccurate or poorly resolved phylogeny reconstruction. Morgan et al. (2014) went so far as to conclude that "neither individual gene datasets nor the SM [concatenated supermatrix] dataset, were able to resolve these four Superorders" or many of the interordinal and interfamily clades. This finding is itself worth re-visiting, since mitogenome analyses routinely recover at least Xenarthra and Afrotheria (e.g. Arnason et al., 2002; Reyes et al., 2004; Kjer and Honeycutt, 2007). Nevertheless, other critiques, including Springer et al. (2001) and Galtier et al. (2009) are well-based in noting that mitogenome trees regularly fail to reconstruct benchmark clades, including Laurasiatheria, Ferae, Euarchontoglires, Glires, Primates, and key groupings within Artiodactyla.

Both Springer et al. (2001) and Morgan et al. (2014) attributed erroneous mtDNA phylogenies to substitution saturation, which erodes phylogenetic signals, and thus increases the influence of non-phylogenetic signals. For example, mammalian mitogenomes exhibit striking variations in base composition across species (Gibson et al., 2005). Phillips and Penny (2003) showed that high thymine content in monotremes and several marsupials contributed to mitogenome phylogenies incorrectly grouping marsupials with monotremes, rather than with placentals. Erinaceids (hedgehogs and allies) share a similar base composition to these monotremes and marsupials, and tend to be "pulled" towards the base of the placental mitogenomic phylogeny, as in Penny and Hasegawa (1997) and Arnason et al. (2002), and upon rooting the tree in Lin et al. (2002) and Arnason et al. (2008). Mitogenome trees that most closely agree with the nuclear consensus have up to now, been facilitated in part by omitting rogue taxa, such as erinaceids (e.g. Campbell and Lapointe, 2011; Wu et al., 2014).

Despite concerns relating to phylogenetic performance, mtDNA remains popular. Ease of amplification from universal primers as well as shotgun sequencing, informativeness, and high copy numbers underpin the utility of mtDNA for environmental DNA assessment (Adams et al., 2019) and often as the only choice for highly degraded ancient DNA (Paijmans et al., 2013). Even as innovations give greater access to

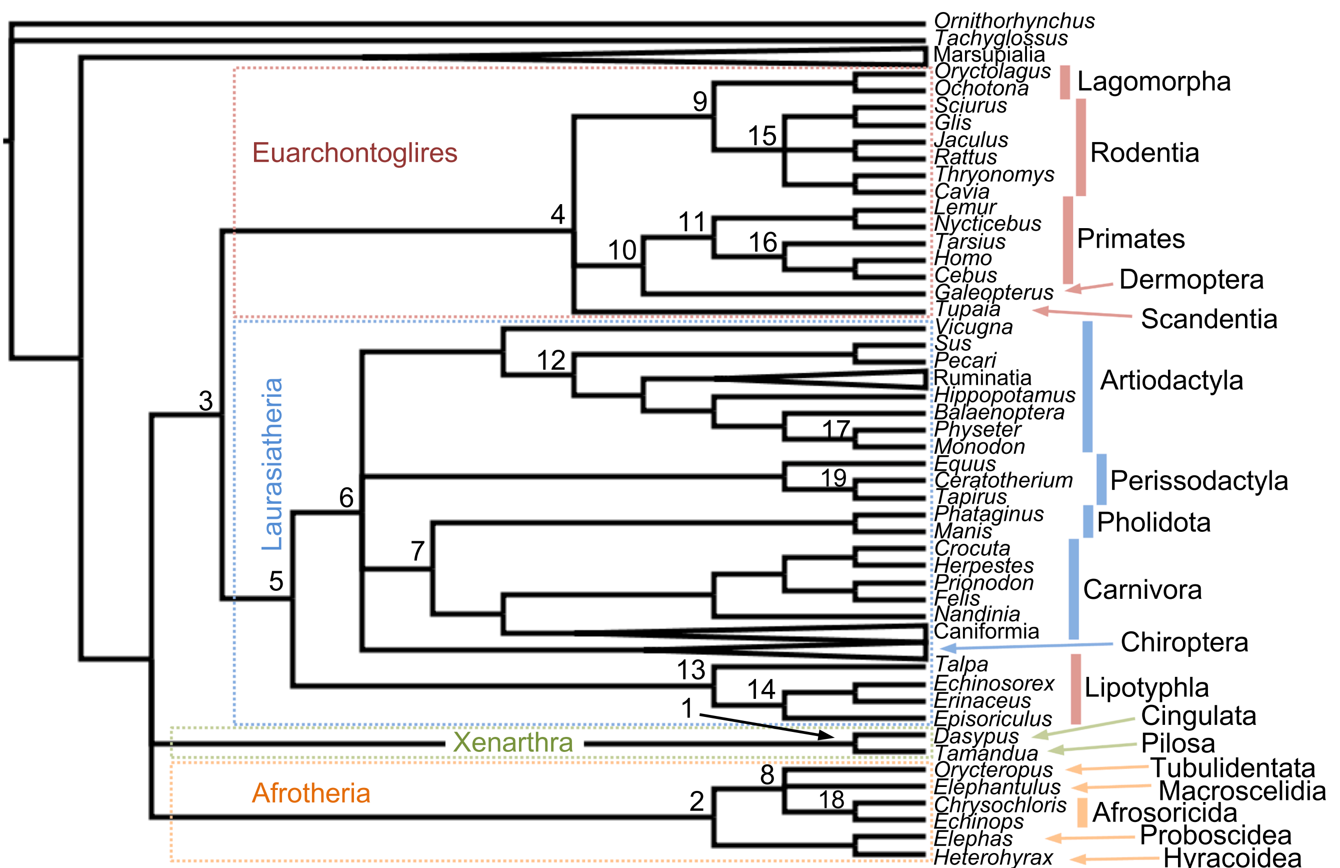


Fig. 1. Constraint phylogeny employed for comparing the 88-taxon mtDNA and nuclear phylogenies against prior expectations. Placentalia is divided into four superorders, Euarchontoglires, Laurasiatheria, Xenarthra and Afrotheria. Numbered clades (1–19) have been considered as phylogenetic reconstruction performance benchmarks and are referable to Fig. 2, S3 and S4. The collapsed clades are expanded in Figure S1.

nuclear DNA the envelope for mtDNA sequencing continues to expand (Tilak et al., 2015; McHugo et al., 2019). Hence, improving phylogeny reconstruction from mtDNA has broad importance.

Early improvements to modelling mtDNA evolution focused on partitioning sites into functional classes, such as protein codon positions and RNA-coding stems and loops to accommodate variation in substitution rates across mitogenomes (e.g. DeBry, 1999; Cao et al., 2000; Reyes et al., 2004). The most commonly used methods for modelling compositional stationarity, such as LogDet (Lockhart et al., 1994) and non-homogenous maximum likelihood (NHML, Galtier and Gouy, 1998) have met with limited success. LogDet in particular is distance-based and has typically been inadequate for accommodating rates across sites variation (but see Cichocki et al., 2015), and in the case of NHML, the assumption of base frequency equivalence (A = T, G = C) that is common to nuclear genomes is strongly violated for mtDNA (Phillips et al., 2010). More general models for accommodating compositional non-stationarity have been developed (e.g. Blanquart and Lartillot, 2008; Jayaswal et al., 2014), but are computationally prohibitive with large datasets.

A method that reduces substitution saturation and compositional bias is RY coding, which groups purines (A,G → R) and pyrimidines (C,T → Y), thus retaining only the slower evolving transversion signal (Woese et al., 1991; Phillips et al., 2001). RY coding has been successful in correcting artefacts of model misspecification among mammals, from within genera (Robins et al., 2010) to between monotremes, marsupials and placentals (Phillips and Penny, 2003). RY coding has also shown promise with recovering interordinal placental mammal relationships (Lin et al., 2002), although as far as we are aware, has not yet been employed to take advantage of recent improvements in mitogenome taxon sampling.

In this study we use base compositional heterogeneity and phylogenetic signal erosion metrics to inform RY coding procedures, and to evaluate the performance of mitogenomes for reconstructing placental mammal phylogeny, relative to concatenated nuclear protein-coding sequences. In particular, we examined the influence of RY coding, improved taxon sampling and partitioning substitution models to accommodate amino acid hydrophobicity and RNA stems and loops. Using the combination of these strategies we re-examine the relationships of three extinct megafaunal taxa for which ancient DNA (aDNA) research has only recovered mtDNA: glyptodonts (Delsuc et al., 2016; Mitchell et al. 2016), the South American ungulate, *Macrauchenia* (Westbury et al., 2017), and sabre-toothed cats (Paijmans et al., 2017). We further show that RY-coded mtDNA can enhance phylogenetic signals in combined analyses alongside nuclear data, for which we consider remaining uncertainties in placental mammal phylogeny.

2. Materials and methods

2.1. DNA sequences

Complete mitochondrial genomes for 175 mammal taxa were obtained from GenBank (accessions are provided in Table S1) and were aligned firstly in MUSCLE (Edgar, 2004) and manually edited in Se-Al v2.0a11 (Rambaut, 1996). These sequences cover all mammalian orders and include 31 marsupials, 141 placentals and three monotremes. The mtDNA was partitioned into the three codon positions for the 13 concatenated protein-coding genes, and as stems and loops for the concatenated rRNAs and tRNAs, giving a total of 14,226 bp after sites with ambiguous homology were removed. Three variants of the mtDNA datasets were prepared: MT_{NT}, with standard coding (A,C,G,T) for all partitions, MT_{3RY}, with only the third codon positions RY-coded, and MT_{RY}, with the third codon positions excluded, but all other sites RY-coded. These datasets reflect increasing effort to remove the most saturated and compositionally heterogeneous sites.

Nuclear DNA sequence alignments were prepared for comparing phylogenetic signals with the mtDNA. The primary nuclear DNA alignment (27,828 bp) included the 21 protein coding genes from Meredith et al. (2011) and followed the same procedure as described above for the mtDNA. The non-protein coding UTRs from Meredith et al. (2011) are missing for many taxa and were not included in our analyses. Several chimeric taxa improve the completeness of the protein coding data (see Table S2), giving an 88-taxon alignment that can be directly compared with a reduced (88-taxon) mitogenomic alignment, with an emphasis on reconstructing phylogenetic performance benchmark clades (see Fig. 1). This near-complete gene sampling is important for reducing biases in comparing nucleotide compositional heterogeneity, phylogenetic signal erosion and phylogenetic resolving power between the mt and nuclear alignments.

2.2. Compositional heterogeneity

A quantitative analysis of nucleotide composition homogeneity between taxa was undertaken using chi-square tests in PAUP* 4.0b10 (Swofford, 2002) and relative composition variability (RCV, Phillips and Penny, 2003). These statistics compared compositional heterogeneity among placentals for each partition of the 88-taxon mitochondrial and nuclear datasets. The X^2-test can indicate the presence of compositional heterogeneity. However, as discussed by Phillips and Penny (2003) the test does not provide a useful measure of the magnitude of heterogeneity, and statistical power depends both on the number of (variable) sites, which differs between the mt and nuclear datasets and between their partitions. The statistical power of the test also depends on the number of character states, which differs between standard nucleotide and RY coding (Phillips and Penny, 2003). Relative compositional variability (RCV) provides an alternative measure of the magnitude of compositional heterogeneity, as the average difference in nucleotide composition across sites, between taxa (Phillips and Penny, 2003):

$$\mathrm{RCV} = \sum_{i=1}^{n}\left(|Ai - A^{*}| + |Ti - T^{*}| + |Ci - C^{*}| + |Gi - G^{*}|\right)\Big/ n.t$$

where, A_i, T_i, C_i and G_i are the frequencies of each nucleotide for the ith taxon. A^*, T^*, C^* and G^* are averages across the n taxa, and t is the number of sites. Uninformative sites were excluded in both X^2 testing and RCV calculation because these sites dilute the compositional heterogeneity.

Base-frequency distances (Phillips et al., 2006) were also calculated on mitochondrial protein 3rd codon positions. These distances are half the sum of absolute frequency differences between taxon pairs for each nucleotide category. So, the pairwise base-frequency (BF) distance between taxa i and j is:

$$\text{BF distance} = \left(|Ai - Aj| + |Ti - Tj| + |Ci - Cj| + |Gi - Gj|\right)/2$$

where, Ai, Ti, Ci, and Gi, and Aj, Tj, Cj and Gj are the frequencies of each nucleotide for the ith and jth taxa, respectively. Dividing by two is appropriate for ME trees on BF distances because a substitution at a site in taxon i that previously had the same base as for taxon j will result in one unit of standard distance, but two units of base-frequency distance (Phillips and Pratt, 2008).

To examine phylogenetic signal erosion (saturation), uncorrected stemminess was calculated as the proportion of minimum evolution tree p-distance contributed by internal branches (Fiala and Sokal, 1985). This metric for substitution saturation was calculated for each mt and nuclear partition for both the standard (NT) and RY-coded data. For consistency, the overall favoured ML topology (88-taxon combined data, MT_{RY}Nuc) was employed for all partitions.

2.3. Phylogenetic analyses

The mammal phylogeny was initially inferred for the three variants of the 88-taxon mtDNA dataset (MT_{NT}88, MT_{3RY}88 and MT_{RY}88), as well

as for the 88-taxon nuclear dataset (Nuc88). Additional phylogenetic analyses were undertaken with the best performing (RY-coded) mtDNA data, either combined with the nuclear data (MT_{RY}Nuc88) or with expanded taxon sampling (MT_{RY}175).

The substitution model categories for each partition were assigned according to the most general available model suggested from among the Akaike Information Criterion (AIC) and hierarchical likelihood ratio test (hLRT) in jModelTest 2.1.10 (Darriba et al., 2012). In each case the substitution models employed for the standard nucleotide partitions were GTR + I + Γ_4. For the RY-coded partitions the binary CF87 + I + Γ_4 (Cavender and Felsenstein, 1987) model was employed for ML, and F81 + I + Γ_4 was employed for Bayesian inference. The nuclear protein-coding codon partitions, mt protein-coding codon partitions and mt RNA stem and loop partitions were modelled separately to accommodate variation in substitution processes. Subsequent analyses further partitioned mtDNA protein-coding sites according to the hydrophobicity of the amino acid residues they encoded on the placental mammal consensus sequence. This followed Rose and Wolfenden's (1993) hydrophobicity scale that was based on 3D models, with Cys, Ile, Leu, Met, Phe, Trp and Val classified as hydrophobic and the remaining amino acids classified as non-hydrophobic.

Bayesian inference analyses were performed in MrBayes v3.2.6 (Ronquist et al., 2012) on XSEDE in the CIPRES Science Gateway portal. The analyses were run with unlinked substitution models and branch-length rate multipliers among each of the nuclear and mtDNA partitions, but with branch lengths fully separate between the nuclear and mtDNA in the combined analysis. Three MCMC chains for each of two independent runs were executed for 20–25 million generations, with trees being sampled every 5,000 generations. The burn-in for each MrBayes run (5–10 million generations) ensured that –ln*L* had plateaued, clade frequencies had converged between runs and estimated sample sizes (ESS) for substitution parameters exceeded 100 (using Tracer v1.6, (Rambaut and Drummond, 2007).

ML analyses were performed in IQ-TREE 1.6.6 (Nguyen et al., 2014) with ultrafast bootstrap approximation (1,000 replicates) and substitution models as described above. Proportional branch length optimization was employed across partitions (-spp option). ML on the combined MT_{RY}Nuc88 dataset estimated fully separate branch lengths for partitions, to allow the mtDNA and nuclear branch lengths to be fully independent. The RY-coded data were treated as binary characters.

Maximum likelihood significance tests were carried out in IQ-TREE, with the favoured unconstrained tree for each dataset being compared with the tree that was favoured when constrained to fit the molecular consensus (Fig. 1). The same partition scheme was applied as above, with each partition modelled separately, including for branch lengths. With only two topologies compared in each case, we report the KH test (Kishino and Hasegawa, 1989) results, although the results for the SH test are identical, since the latter test collapses to the former for comparisons between only two trees.

To better understand the extent to which phylogenetic signal erosion and compositional non-stationarity might distort phylogenetic inference we inferred the 88-taxon phylogeny from mtDNA protein-coding 3rd codon position transitions alone. These sites are the most saturated and compositionally heterogeneous (Table 1). Exclusion of transversion signal is not suited to ML and Bayesian inference, because transversion probabilities are integral to calculating site likelihoods. Therefore, a minimum evolution tree based on transitions only was inferred on mtDNA protein-coding 3rd positions (in PAUP* 4.0b10).

3. Results and discussion

3.1. How accurate are mitogenome phylogenies for placental mammals?

The accuracy of published mitogenomic phylogenies for ordinal and deeper-level relationships among placental mammals varies considerably, when judged against the recent nuclear consensus shown in Fig. 1.

Table 1
Phylogenetic signal retention (stemminess) and base compositional homogeneity (X^2 P-value and relative composition variability, RCV), calculated on the 88-taxon mitochondrial (standard NT and RY-coded) and nuclear datasets.

	Stemminess	Composition X^2 P-value	RCV	Stemminess /RCV
mtDNA NT-coding				
Protein codon 1	0.0678	<0.0001	0.0628	1.0796
Protein codon 2	0.0869	0.8915	0.0343	2.5335
Protein codon 3	0.0302	<0.0001	0.1161	0.2601
RNA stems	0.0951	0.9814	0.0386	2.4637
RNA loops	0.0761	0.0132	0.0666	1.1426
mtDNA RY-coding				
Protein codon 1	0.1300	0.0923	0.0257	5.0584
Protein codon 2	0.1138	1.000	0.0210	5.4190
Protein codon 3	0.0800	<0.0001	0.0553	1.4467
RNA stems	0.1682	1.000	0.0190	8.8526
RNA loops	0.1333	0.9208	0.0391	3.4092
Nuclear DNA (NT-coding)				
Protein codon 1	0.1688	0.4771	0.0195	8.6564
Protein codon 2	0.1704	0.1940	0.0228	7.4737
Protein codon 3	0.1489	<0.0001	0.0326	4.5675

The most extreme cases, however, are anomalous. In particular, Morgan et al.'s (2014) primary supermatrix phylogeny was unable to recover numerous key superordinal, interordinal and ordinal level relationships (see Fig. 2e). They concluded that mtDNA was unsuitable for inferring placental relationships. However, closer inspection reveals that these errors are contributed to by the supermatrix including numerous poorly gene-sampled taxa. For example, the hedgehog, *Hemiechinus* was sampled for only two of the 13 mt proteins and was reconstructed as sister to all other placentals, while non-overlapping gene sampling left the elephant shrews polyphyletic. Morgan et al.'s (2014) reported carnivoran paraphyly (including the bat, *Sturnia*) appears to have been induced by one of only two included protein sequences for the bat being an incorrectly labelled *Canis* Cyt*b* sequence (Q35873, 97% similarity to *Canis latrans*, ≤ 88% similarity to other bats). Indeed, upon excluding the incorrectly or undersampled *Hemiechinus*, *Macroscelides* and *Sturnira* from Morgan et al.'s (2014) supermatrix analysis (Fig. 2f), their tree is closely comparable to our own 88-taxon NT or 3RY trees (Fig. 2g–j).

At the optimistic end of the accuracy spectrum, the mitogenome phylogenies of Campbell and Lapointe (2011) and Wu et al. (2014) recovered all of the superordinal and more of the interordinal and shallower clades than other published studies shown in Fig. 2 a–f. However, both of the aforementioned studies excluded "rogue" taxa that are frequently incorrectly placed and may adversely affect other relationships in the tree. Both Campbell and Lapointe (2011) and Wu et al. (2014) excluded the hedgehogs (Erinaceidae) and the former also excluded pangolins and the rodent *Anomalurus*, while the latter study additionally excluded tapirs (Tapiridae). As such, we do not consider that these studies are directly comparable with those included in Fig. 2.

Since one of the major benefits of mitogenomes is greater access to ancient DNA than can often be provided by long nuclear sequences, it would be inappropriate to gauge the accuracy of mtDNA phylogeny with the luxury of excluding "rogue" taxa. Thus our baseline for investigating the value of proposed improvements to mtDNA phylogenetic inference against the nuclear consensus is our own 88-taxon analyses with standard nucleotide (NT) coding (Fig. 2g,h) and the similarly sampled studies (Fig. 2c,d) of Gibson et al. (2005) and Kjer and Honeycutt

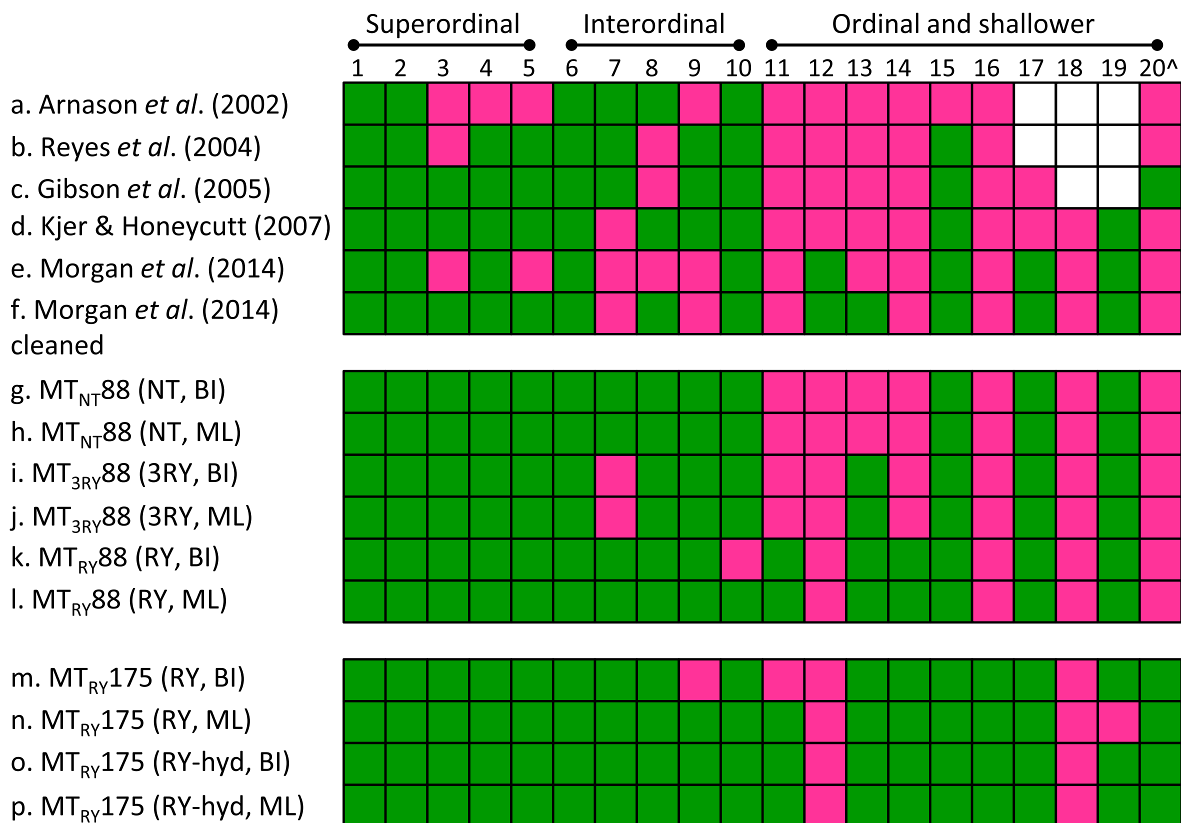


Fig. 2. Summary of mitogenome phylogenetic performance in correctly reconstructing (green) or not reconstructing (magenta) placental mammal clades in previous studies (a-f) and under alternative strategies used in the present study for the 88-taxon dataset (g-l) and for the expanded, 175-taxon dataset (m-p). The numbered superordinal, interordinal and the ordinal and shallower clades (1–19) are labelled in Fig. 1. Blank squares indicate that sampling exclusion precludes evaluation. Abbreviations among the descriptors for our 88 and 175-taxon analyses: BI; Bayesian inference (MrBayes), -hyd; hydrophobicity partitioning, ML; maximum likelihood (IQ-TREE), NT; standard nucleotide coding, 3RY; 3rd codon positions are RY coded, RY; all sites are RY coded (3rd codon positions are deleted). ^Clade 20 is unlabelled in Fig. 1 and includes all rodents except Sciuromorpha. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(2007), all of which include "rogue" taxa. Each of these baseline studies resolved all four placental superorders, Xenarthra, Afrotheria, Laurasiatheria and Euarchontoglires, as well as Boreoeutheria and other well-established interordinal relationships, except in some cases, Ferae (pangolins and carnivorans) and the African insectivore clade, Afroinsectiphilia. The relationships that are the focus of Fig. 2 do not include clades that are either routinely recovered in all analyses (such as Paenungulata) or that remain contentious even with nuclear genome-scale datasets, such as the scrotiferan polytomy (Artiodactyla, Perissodactyla, Ferae and Chiroptera).

The support we find for deep-level placental relationships should dispel some of the concerns that have been levelled against mtDNA (e.g. Springer et al., 2001; Morgan et al., 2014). Even before RY coding or extending the taxon sampling our 88-taxon analyses recovered all four superordinal clades and Boreoeutheria at $\geq$ 96% ML-BP and 1.00 BPP. Contrary to the received wisdom of mammalian mitochondrial phylogenetics it is shallower-level relationships that remain the most recalcitrant in our 88-taxon MT_{NT} analyses and in other recently published studies (Fig. 2, clades 11–14, 16, 18, 20). Examples include hedgehogs being excluded from other lipotyphlans (ML-BP 100%, BPP 1.00), primate paraphyly (colugo grouping with anthropoid primates: ML-BP 88%, BPP 1.00), and tarsiers grouping with strepsirrhines (ML-BP 83%, BPP 0.75) instead of their well-established placement with anthropoids (e.g. Schmitz et al., 2016; Liu et al., 2017). Understanding the basis for these conflicts with the nuclear consensus and identifying data treatments or models that recover the accepted relationships is important for instilling confidence in ancient DNA mitogenomics.

3.2. *Phylogenetic signal erosion and nucleotide compositional heterogeneity*

Substitution saturation has been the prime suspect for explaining the poor phylogenetic performance of mitogenomes in comparison with nuclear DNA (Springer et al., 2001; Galtier et al., 2009). Thus, we calculated uncorrected stemminess, which is the proportion of phylogenetic signal (tree length) retained on internal branches. The results shown in Table 1 broadly reflect previous investigations of saturation in mammals (e.g. Matthee and Davis, 2001: Reyes et al., 2004; Gibson et al., 2005). With standard nucleotide coding, phylogenetic signal retention is far lower for the mtDNA partitions (stemminess: 0.0302–0.0951) than for the nuclear DNA partitions (stemminess: 0.1489–0.1704). Phylogenetic signal erosion alone has some explanatory power for erroneous mitogenome phylogenies. This is evidenced by the particularly poor performance of maximum parsimony (MP, see Figure S2) relative to ML and Bayesian inference, which are effective in correcting for saturation, except in extreme cases (Hendy and Penny, 1989; Philippe et al., 2005). In particular, the fastest evolving mitogenomes, the hedgehogs (erinaceids) and rodents fell outside all other placentals under MP, but were brought back into agreement with the modern nuclear consensus (Fig. 1) under ML and Bayesian inference, at least in so much as grouping within Laurasiatheria and Euarchontoglires, respectively.

Mitogenome phylogenetic conflicts with the nuclear consensus may often stem from synergistic artefacts of saturation and compositional non-stationarity, as has been suggested for cases involving monotremes and marsupials (Phillips and Penny 2003; Phillips et al., 2006). For the

present data, the base composition X^2 test indicated significant ($P < 0.05$) heterogeneity for standard (NT) coding among the mitochondrial 1st and 3rd codon positions, mt RNA loop sites and also for the nuclear 3rd codon positions. However, the power of the test is sensitive to the number of variable characters sampled and hence, we instead use the relative compositional variability (RCV) metric to compare the magnitude of base compositional heterogeneity between partitions. All of the mt partitions have higher RCV than each of the nuclear partitions (Table 1). Moreover, with the expression of compositional biases being facilitated by phylogenetic signal erosion, it is the stemminess/RCV metric that most clearly shows the potential for these biases to mislead phylogeny reconstruction. All of the NT-coded mt partitions have far lower stemminess/RCV than do the nuclear partitions (Table 1). This is most extreme for the 3rd codon positions, with stemminess/RCV of 0.2601 for the mt 3rd codon positions, compared with 4.5675 for nuclear 3rd codon positions.

It is well-established that mt protein-coding 3rd codon positions contribute the most variable sites and branch length to mammalian mitogenomic phylogeny, and among these sites the highest rate of evolution is attributable to transitions (Hasegawa et al., 1990; Saccone et al., 2000). Minimum evolution on these mtDNA 3rd codon position transitions reconstructed a tree (Fig. 3) that has little resemblance to the consensus phylogeny, but widely disperses members of superorders across the tree. The relationships instead closely align with 3rd codon position cytosine/thymine content, including highly anomalous groupings of distantly related taxa, such as the musky rat kangaroo (*Hypsiprymnodon*), human (*Homo*), pangolin (*Manis*) and sperm whale (*Physeter*) all with high cytosine content, and conversely, opossums (*Didelphis*, *Caluromys*), hedgehogs (*Erinaceus*, *Echinosorex*), dormouse (*Glis*) and fruit-eating bat (*Artibeus*) with very low cytosine content.

RY coding the mt protein coding 3rd codon sites substantially increases stemminess/RCV (Table 1), but to a level that is still well below the nuclear partitions. RY coding the mitochondrial 1st and 2nd codon positions and the RNA stem and loop sites is more promising, with stemminess/RCV closely comparable to the nuclear partitions (Table 1). It has been argued that excluding mt 3rd positions (or RY coding, which excludes transitions) removes the most variable sites, thereby increasing susceptibility to stochastic variation (Ishikawa et al., 2012). A counterpoint to this argument is that phylogenetic inference from the remaining variation should be less susceptible to model misspecification associated with phylogenetic signal erosion and compositional heterogeneity. In the following section, we consider previous efforts to navigate this precision-accuracy trade-off and we investigate the influence of RY coding, hydrophobicity partitioning and expanded taxon sampling for reconstructing placental mammal phylogeny from mitogenomes.

3.3. Rescuing mitogenome phylogeny

The common pattern of error in mitogenome phylogeny reconstruction is misplacement of long branches. Historically this provided

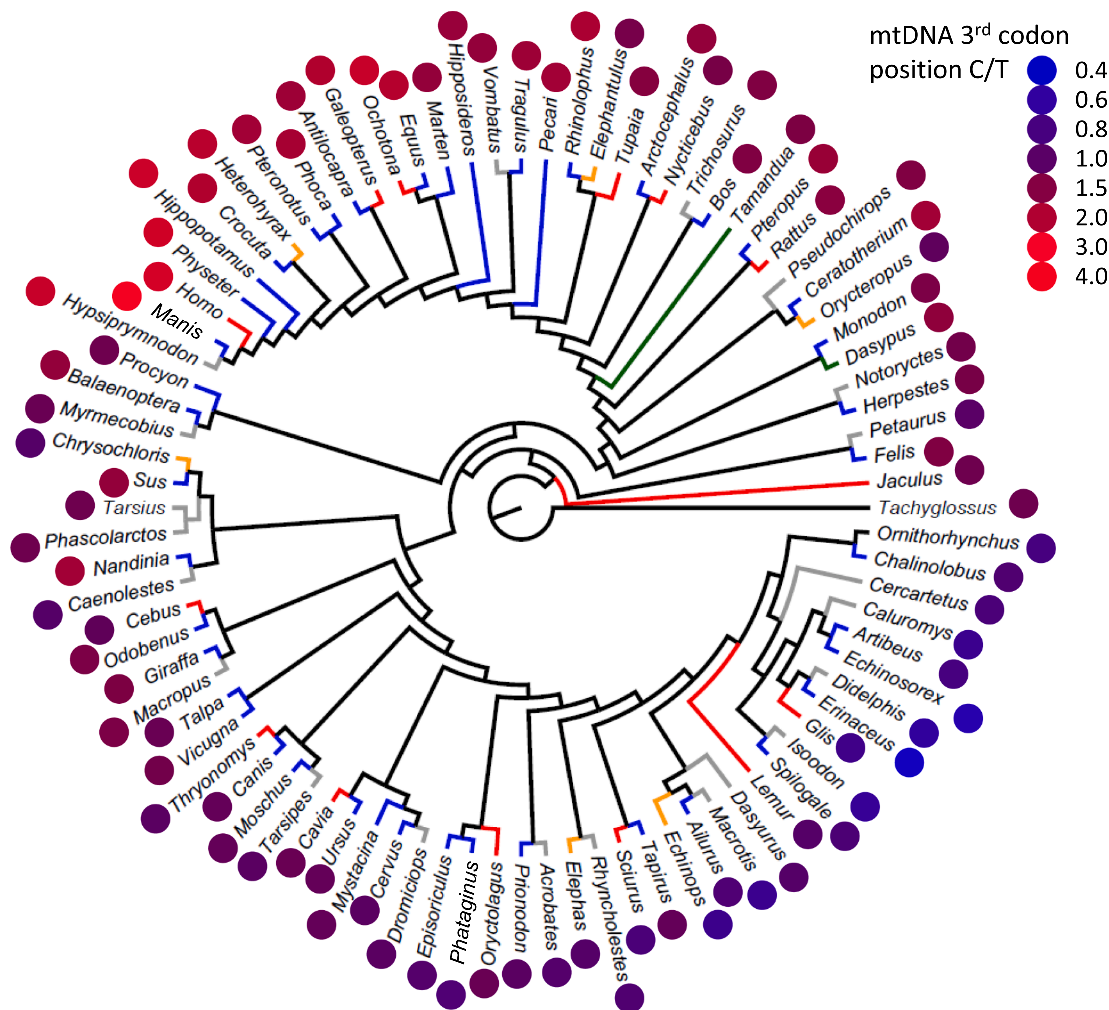


Fig. 3. Minimum evolution tree on mtDNA protein-coding 3rd codon positions, based on transitions only. Clades are reconstructed in close agreement with base composition (especially cytosine/thymine content). External branches are coloured according to superordinal affinities: Euarchontoglires (red), Laurasiatheria (blue), Afrotheria (orange), Xenarthra (green), along with marsupials (grey) and monotremes (black).

infamous conflicts with well-established clades, such as D'Erchia et al. (1996) inferring rodent paraphyly, as well as incongruence with the nuclear consensus on the superordinal relationships of placental mammals. The deeper-level divergences have since been largely brought into agreement with the nuclear consensus upon improved taxon sampling breaking up long mitochondrial branches (e.g. Gibson et al., 2005; Kjer and Honeycutt, 2007; and see Fig. 2 g,h). However, similar problems remain shallower in the mitogenome tree, such as the fast evolving erinaceids (hedgehogs) falling outside other laurasiatherians and the colugo (*Galeopterus*, Dermoptera) grouping with anthropoids, rendering Primates paraphyletic. Both cases present the synergistic problem of phylogenetic signal erosion along long branches and accumulation of nucleotide composition bias.

The stemward drift of the erinaceids in mtDNA trees aligns with their low C/T ratio being similar to outgroup marsupials (Fig. 3). As a measure of compositional similarity, the base frequency distance at 3rd codon positions between erinaceids and all other included taxa is closest to the marsupials, *Didelphis* (0.0378) and *Isoodon* (0.0420) and more than twice that distance to the erinaceid's expected sister, the shrew, *Episoriculus* (0.0853). Conversely, the colugo, *Galeopterus* and the anthropoid primate, *Homo* share high C/T ratios (Fig. 3) and their base frequency distance (0.0450) is half that of *Galeopterus* to non-anthropoid archontans (0.0902).

First, we investigated whether employing RY coding could correctly resolve mitogenomic phylogeny, even without additional taxon sampling. This is especially important for aDNA research, which often lends little opportunity to extend taxon sampling. RY coding only the mt protein 3rd codon positions did not substantially improve phylogeny reconstruction. The same number (seven) of the 20 key clades are not recovered, as for standard MT_{NT} coding (Fig. 2g–j), and ML hypothesis KH testing against the nuclear consensus constraint tree rejects the MT_{3RY} tree ($P = 0.0190$) almost as strongly as for the MT_{NT} tree ($P = 0.0070$). However, stemminess and relative compositional variability (Table 1) suggest that a more appropriate solution is to entirely exclude 3rd codon positions and RY code all of the remaining mtDNA partitions. This stringent treatment is more promising; the resulting MT_{RY} tree was only marginally rejected ($P = 0.0494$), and both the erinaceid and colugo placements were reconstructed in agreement with the nuclear consensus, respectively as sister to the shrews (Soricidae) and as sister to monophyletic Primates (Fig. 2k,l).

RY coding is a rather blunt instrument and some phylogenetic signal is lost. However, less stringent treatments, such as using the protein (amino acid) sequence (e.g. Waddell et al., 1999; Morgan et al., 2014) or excluding 3rd and AGY coding 1st codon positions (Gibson et al., 2005) have likely been less successful, in part due to substantial compositional bias and phylogenetic signal erosion still being present. Employing the amino acid sequence in the present case recovers fewer benchmark clades than does our more stringent RY-coded DNA treatment (Figure S3). Phillips and Penny (2003) found a similar level of stemminess/RCV for mammalian mt protein (amino acid) sequences and both NT coded 1st codon and RNA loop positions. Here for the 88-taxon mt data we note that AGY coding 1st codon positions still leaves stemminess/RCV at 2.71, comparable with NT coded 2nd codon and RNA stem positions (cf. Table 1). Ultimately, however, improving the computational efficiency of non-stationary models that explicitly model heterogeneity in substitution processes across lineages, such as CAT-GTR (Lartillot et al., 2009) or HAL-HAS (Jayaswal et al., 2014) will hopefully facilitate accurate phylogeny reconstruction for large datasets, without discarding evolutionary information.

For now, the computational simplicity of RY coding allows extensive taxon sampling and complex partitioning. We take advantage of this by expanding taxon sampling to 175 taxa and performing phylogenetic analyses with and without the remaining 1st and 2nd protein codon partitions being further partitioned into hydrophobic and non-hydrophobic sites (see Fig. 2 m-p). The reconstructed ML trees do not significantly differ from the nuclear consensus constraint tree in KH testing either with hydrophobicity partitioning ($P = 0.1150$) or without ($P = 0.0823$). These analyses all improve on the 88-taxon analyses by recovering the well-established tarsier-anthropoid primate clade and the rodent root (sciuromorphs as sister to other rodents) that has been established by retroposons (Churakov et al., 2010), morphology (e.g. Wible et al., 2005; O'Leary et al., 2013) and most genome-scale nuclear sequence analyses (e.g. Morgan et al., 2014; Romiguier et al., 2013; Liu et al., 2017).

Hydrophobicity partitioning has been employed in some mitogenomic analyses at least since Naylor and Brown (1997) and DeBry (1999). But here, in combination with RY coding the 175-taxon hydrophobicity-partitioned analyses provided better agreement with the nuclear consensus than all previous mtDNA analyses and each of the five longest (1,551–4,260 bp) included nuclear genes (Figure S4). Indeed, this mtDNA treatment is comparable in performance to analysis of the 88-taxon nuclear data re-sampled to sequence length equivalence with the mtDNA (Figure S5). The combination of RY coding and hydrophobicity partitioning might also be valuable beyond mitogenomics for reconstructing far deeper-level nuclear or genomic phylogenies, where phylogenetic signal erosion and compositional biases are also prominent (e.g. Philippe et al., 2011).

The only remaining, putatively incorrect placements with the hydrophobicity-partitioned analyses are single branch steps away from the accepted placements; the tenrec, *Echinops* grouped with the aardvark (*Orycteropus*) instead of golden moles (chrysochlorids), and camelids swapped with pigs (suids) at the base of Artiodactyla. *Echinops* in particular is a very long branch (Fig. 4), and the history of breaking up long branches in mitogenomic phylogeny suggests that including another deeply diverging tenrecid, such as *Potamogale*, will likely provide correction. In the case of camels and pigs, their successive divergences from other artiodactyls may be as little as two million years (Phillips and Fruciano, 2018). Hence, their placements may even represent the true mtDNA gene-tree, since introgression and incomplete lineage sorting are plausible explanations for mitogenomic incongruence over such short timescales (e.g. Doronina et al., 2015; Nilsson et al., 2018).

3.4. Ancient mtDNA and insights into placental mammal evolution

Mitogenomes have recently been published for three extinct Pleistocene megafaunal taxa that have uncertain affinities based on morphology and no available nuclear DNA sequences. Each of these published studies was based on standard NT coded sequences, and thus, they may be particularly susceptible to compositional non-stationarity and long-branch artefacts. We investigate the relationships of these megafaunal taxa, using RY coding and hydrophobicity partitioning (Fig. 4).

The South American litoptern ungulate, *Machrauchenia* was found by Westbury et al. (2017) to be sister to odd-toed ungulates, such as horses and rhinos. We found the same relationship, and although statistical support is moderate (BPP 0.77, ML-BP 73%), another study that sequenced ancient collagen amino acid residues (Welker et al., 2015) also placed *Machrauchenia* and *Toxodon* as sister to perissodactyls. Considered together, these results substantiate a close link between South American ungulates and perissodactyls, and in turn provide vital evidence for understanding Early Tertiary mammalian biogeography.

Also from South America, the two-tonne, club-tailed glyptodont, *Doedicurus* has recently been nested well within armadillos, possibly as sister to both Tolypeutinae and Chlamyphorinae (Delsuc et al., 2016; Mitchell et al., 2016). Our analyses confirm the placement of glyptodonts with the armadillo family, Chlamyphoridae (Fig. 4), either in the arrangement noted above (for ML) or as sister to all three sub-families, Euphractinae, Tolypeutinae and Chlamyphorinae (Bayesian inference). The sabretooth cat, *Smilodon populator* is a putative predator of both *Macrauchenia* and *Doedicurus* (at least as juveniles) and together with the scimitar cat (*Homotherium*), these machirodontines have been placed as

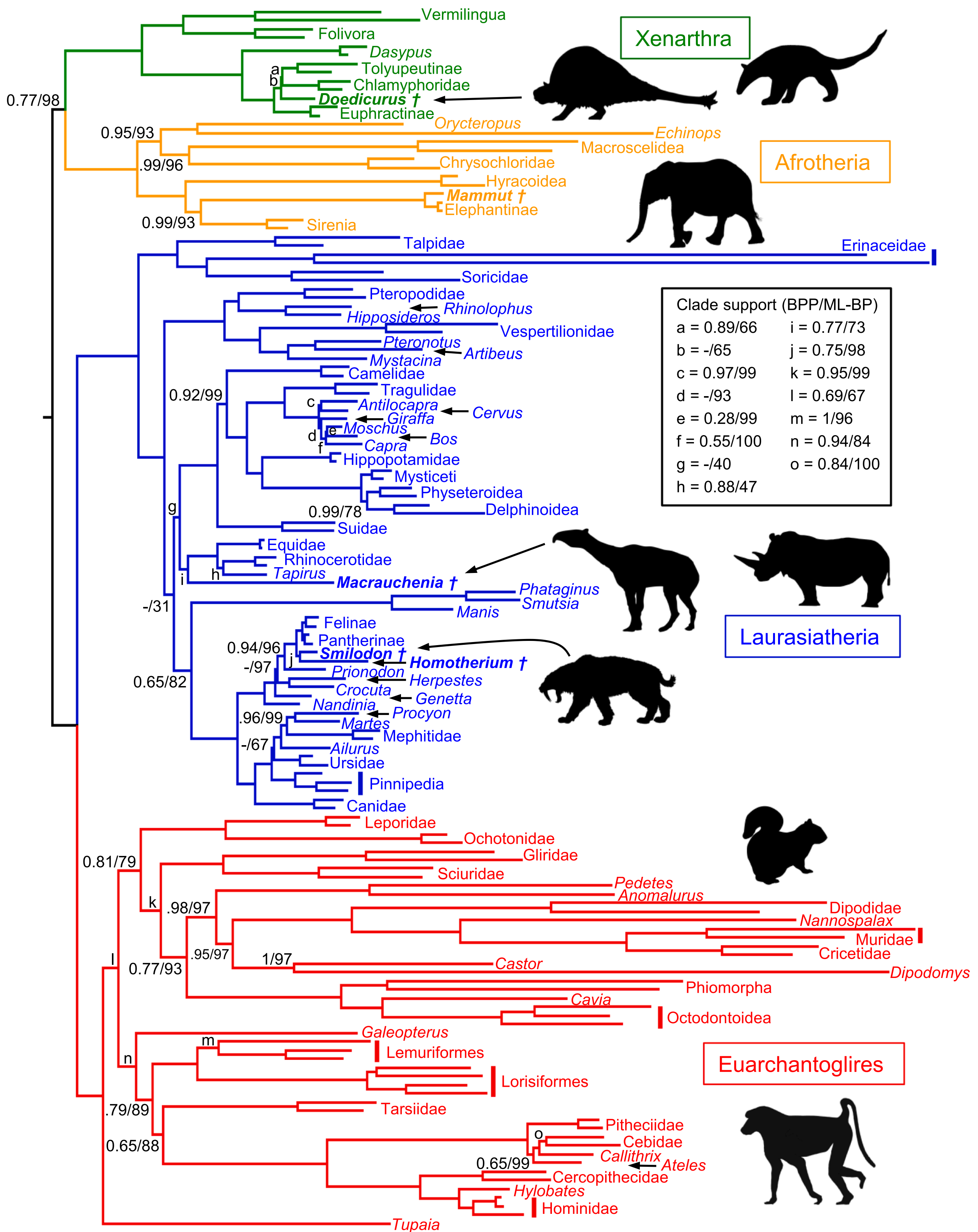


Fig. 4. IQ-TREE maximum likelihood phylogeny for placental mammals for the 175-taxon mtDNA RY-coded dataset ($MT_{RY}175$) partitioned by codon positions and hydrophobicity for protein-coding data, and stems and loops for RNA-coding data. Bayesian inference BPP/maximum likelihood BP support for clades (if < 98%) is shown at nodes or referred to the box (a-o). The tree is rooted with marsupial and monotreme outgroup taxa (see Supplementary material). Images from phylopic.org: anteater (Xavier Jenkins), *Doedicurus* (Becky Barnes), elephant, *Smilodon*, *Macrauchenia* (Steven Traver), rhinoceros (Oscar Sanisidro), squirrel (Anthony Caravaggi), baboon (Owen Jones).

sister to "modern" Felinae cats in analyses of partial (Barnett et al., 2005) and near-complete mitogenomes (Paijmans et al., 2017). Our analyses strongly endorse this relationship (Fig. 4).

Beyond the inclusion of aDNA, previous authors have noted that mtDNA often performs well in combination with nuclear DNA (Murphy et al., 2001a; Fisher-Reid and Wiens, 2011). Here, combined analysis of mtDNA with 3-fold longer nuclear data either maintained or improved upon the nuclear support for all generally accepted clades (Figure S6). This includes groupings that mtDNA alone did not favour, thus indicating "hidden support". The upshot of this finding is that appropriately treated mtDNA in combined analyses may generally improve phylogenetic inference. This lends additional confidence to resolving some of the most contentious nodes in the tree. Our ML and Bayesian combined data analyses strongly favour Ferungulata (carnivorans, pangolins, perissodactyls and artiodactyls) and increase support for rooting the placental mammal tree between the ostensibly northern and southern originating clades, respectively Boreoeutheria and Atlantogenata.

3.5. Conclusion

The combination of improved taxon sampling, RY coding and hydrophobicity partitioning endows mammalian mitogenomes with phylogenetic inference accuracy almost on par with a 21-gene nuclear dataset, although with less statistical power. Here, Mark Twain's often misquoted, "The report of my death was an exaggeration" seems appropriate for mitogenomic phylogeny, especially in view of the prominent role mtDNA can play in reconstructing evolution from ancient DNA (e.g. Mitchell et al., 2014; Cascini et al., 2019). However, taxa on long, unbroken branches, such as the aardvark remain difficult to correctly place with mitogenomes, and particular caution should be afforded for phylogenetic inferences of rapidly diversifying (often shallower) taxa where mtDNA phylogeny may reflect deep coalescence or introgression.

CRediT authorship contribution statement

Matthew J. Phillips: Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Sarah Shazwani Zakaria:** Formal analysis, Data curation, Writing - original draft.

Declaration of competing interests

The authors declare that the research was undertaken without any commercial or financial relationships that could be considered a potential conflict of interest.

Acknowledgements

We thank Andrew Baker, Peter Prentis and two anonymous reviewers for valuable comments on the manuscript.

Funding

This work was supported by a Discovery Project grant to MJP from the Australian Research Council (DP150104659).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107082.

References

Adams, C.I., Knapp, M., Gemmell, N.J., Jeunen, G.-J., Bunce, M., Lamare, M.D., Taylor, H.R., 2019. Beyond biodiversity: can environmental DNA (eDNA) cut it as a population genetics tool? Genes 10, 192.
Arnason, U., Adegoke, J.A., Bodin, K., Born, E.W., Esa, Y.B., Gullberg, A., Nilsson, M., Short, R.V., Xu, X., Janke, A., 2002. Mammalian mitogenomic relationships and the root of the eutherian tree. Proc. Natl. Acad. Sci. 99, 8151–8156.
Arnason, U., Adegoke, J.A., Gullberg, A., Harley, E.H., Janke, A., Kullberg, M., 2008. Mitogenomic relationships of placental mammals and molecular estimates of their divergences. Gene 421, 37–51.
Ballard, J.W.O., Whitlock, M.C., 2004. The incomplete natural history of mitochondria. Mol. Ecol. 13, 729–744.
Barnett, R, Barnes, I, Phillips, M.J., Martin, L.D., Harrington, C.R., Leonard, J.A., Cooper, A, 2005. Evolution of the extinct Sabretooths and the American cheetah-like cat. Curr. Biol. 15, R589–R590.
Blanquart, S., Lartillot, N., 2008. A site- and time-heterogeneous model of amino acid replacement. Mol. Biol. Evol. 25, 842–858.
Campbell, V., Lapointe, F.-J., 2011. Retrieving a mitogenomic mammal tree using composite taxa. Mol. Phylogenet. Evol. 58, 149–156.
Cao, Y., Fujiwara, M., Nikaido, M., Okada, N., Hasegawa, M., 2000. Interordinal relationships and timescale of eutherian evolution as inferred from mitochondrial genome data. Gene 259, 149–158.
Cascini, M., Mitchell, K.J., Cooper, A.J., Phillips, M.J., 2019. Reconstructing the evolution of giant extinct kangaroos: comparing the utility of DNA, morphology, and total evidence. Syst. Biol. 68, 520–537.
Cavender, J.A., Felsenstein, J, 1987. Invariants of phylogenies in a simple case with discrete states. J. Classif. 4, 57–71.
Churakov, G., Sadasivuni, M.K., Rosenbloom, K.R., Huchon, D., Brosius, J., Schmitz, J., 2010. Rodent evolution: back to the root. Mol. Biol. Evol. 27, 1315–1326.
Cichocki, A., Cruces, S., Amari, S.-I., 2015. Log-determinant divergences revisited: alpha-beta and gamma log-det divergences. Entropy 17, 2988–3034.
Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Meth. 9, 772 772.
DeBry, R.W., 1999. Maximum likelihood analysis of gene-based and structure-based process partitions, using mammalian mitochondrial genomes. Syst. Biol. 48, 286–299.
Delsuc, F., Gibb, G.C., Kuch, M., Billet, G., Hautier, L., Southon, J., Rouillard, J.-M., Fernicola, J.C., Vizcaíno, S.F., MacPhee, R.D., 2016. The phylogenetic affinities of the extinct glyptodonts. Curr. Biol. 26, R155–R156.
D'Erchia, A.M., Gissi, C., Pesole, G., Saccone, C., Arnason, U., 1996. The guinea-pig is not a rodent. Nature 381, 597–600.
Doronina, L., Churakov, G., Shi, J., Brosius, J., Baertsch, R., Clawson, H., Schmitz, J., 2015. Exploring massive incomplete lineage sorting in arctoids (Laurasiatheria, Carnivora). Mol. Biol. Evol. 32, 3194–3204.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.
Fiala, K.L., Sokal, R.R., 1985. Factors determining the accuracy of cladogram estimation: evaluation using computer simulation. Evolution 39, 609–622.
Fisher-Reid, M.C., Wiens, J.J., 2011. What are the consequences of combining nuclear and mitochondrial data for phylogenetic analysis? Lessons from *Plethodon* salamanders and 13 other vertebrate clades. BMC Evol. Biol. 11, 300.
Funk, D.J., Omland, K.E., 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34, 397–423.
Galtier, N., Gouy, M., 1998. Inferring pattern and process: maximum likelihood implementation of a nonhomogeneous model of DNA sequence evolution for phylogenetic analysis. Mol. Biol. Evol. 15, 871–879.
Galtier, N., Nabholz, B., GlÉmin, S., Hurst, G.D.D., 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Mol. Ecol. 18, 4541–4550.
Gibson, A., Gowri-Shankar, V., Higgs, P.G., Rattray, M., 2005. A comprehensive analysis of mammalian mitochondrial genome base composition and improved phylogenetic methods. Mol. Biol. Evol. 22, 251–264.
Hallström, B.M., Janke, A., 2010. Mammalian evolution may not be strictly bifurcating. Mol. Biol. Evol. 27, 2804–2816.
Härlid, A., Arnason, U., 1999. Analyses of mitochondrial DNA nest ratite birds within the Neognathae: supporting a neotenous origin of ratite morphological characters. Proc. Roy. Soc. Lond. B Biol. Sci. 266, 305–309.
Hasegawa, M., Kishino, H., Hayasaka, K., Horai, S., 1990. Mitochondrial DNA evolution in primates: transition rate has been extremely low in the lemur. J. Mol. Evol. 31, 113–121.
Havird, J.C., Sloan, D.B., 2016. The roles of mutation, selection, and expression in determining relative rates of evolution in mitochondrial versus nuclear genomes. Mol. Biol. Evol. 33, 3042–3053.
Hendy, M., Penny, D., 1989. A framework for the quantitative study of phylogenetic data. Syst. Zool. 38, 297–309.
Ishikawa, S.A., Inagaki, Y., Hashimoto, T., 2012. RY-coding and non-homogeneous models can ameliorate the maximum-likelihood inferences from nucleotide sequence data with parallel compositional heterogeneity. Evol. Bioinformat. 8, 357–371.
Janke, A., Xu, X., Arnason, U., 1997. The complete mitochondrial genome of the wallaroo *(Macropus robustus*) and the phylogenetic relationship among Monotremata, Marsupialia, and Eutheria. Proc. Natl. Acad. Sci. USA 94, 1276–1281.
Jayaswal, V., Wong, T.K.F., Robinson, J., Poladian, L., Jermiin, L.S., 2014. Mixture models of nucleotide sequence evolution that account for heterogeneity in the substitution process across sites and across lineages. Syst. Biol. 63, 726–742.

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data and the branching order Hominidae. J. Mol. Evol. 29, 170–179.

Kjer, K.M., Honeycutt, R.L., 2007. Site specific rates of mitochondrial genomes and the phylogeny of eutheria. BMC Evol. Biol. 7, 8.

Kriegs, J.O., Churakov, G., Kiefmann, M., Jordan, U., Brosius, J., Schmitz, J., 2006. Retrotransposed elements as archives for the evolutionary history of placental mammals. PloS Biol. 4, e91.

Lartillot, N., Lepage, T., Blanquart, S., 2009. PhyloBayes 3: a bayesian software package for phylogenetic reconstruction and molecular dating. Bioinformatics 25, 2286–2288.

Lin, Y.H., McLenachan, P.A., Gore, A.R., Phillips, M.J., Ota, R., Hendy, M.D., Penny, D., 2002. Four new mitochondrial genomesk and the increased stability of evolutionary trees of mammals from improved taxon sampling. Mol. Biol. Evol. 19, 2060–2070.

Liu, L., Zhang, J., Rheindt, F.E., Lei, F.M., Qu, Y.H., Wang, Y., Zhang, Y., Sullivan, C., Nie, W.H., Wang, J.H., Yang, F., Chen, J., Edwards, S.V., Meng, J., Wu, S., 2017. Genomic evidence reveals a radiation of placental mammals uninterrupted by the KPg boundary. Proc. Natl. Acad. Sci. USA 114, E7282–E7290.

Lockhart, P.J., Steel, M., Hendy, M., Penny, D., 1994. Recovering evolutionary trees under a more realistic model of sequence evolution. Mol. Biol. Evol. 11, 605–612.

Madsen, O., Scally, M., Douady, C.J., Kao, D.J., DeBry, R.W., Adkins, R., Amrine, H.M., Stanhope, M.J., de Jong, W.W., Springer, M.S., 2001. Parallel adaptive radiations in two major clades of placental mammals. Nature 409, 610–614.

Matthee, C.A., Davis, S.K., 2001. Molecular insights into the evolution of the family Bovidae: a nuclear DNA perspective. Mol. Biol. Evol. 18, 1220–1230.

Melo-Ferreira, J., Boursot, P., Carneiro, M., Esteves, P.J., Farelo, L., Alves, P.C., 2012. Recurrent introgression of mitochondrial DNA among hares (*Lepus spp.*) revealed by species-tree inference and coalescent simulations. Syst. Biol. 61, 367–381.

McHugo, G.P., Dover, M.J., MacHugh, D.E., 2019. Unlocking the origins of domestic animals using ancient DNA and paleogenomics. BMC Biol. 17, 98.

Meredith, R.W., Janečka, J.E., Gatesy, J., Ryder, O.A., Fisher, C.A., Teeling, E.C., Goodbla, A., Eizirik, E., Simão, T.L.L., Stadler, T., Rabosky, D.L., Honeycutt, R.L., Flynn, J.J., Ingram, C.M., Steiner, C., Williams, T.L., Robinson, T.J., Burk-Herrick, A., Westerman, M., Ayoub, N.A., Springer, M.S., Murphy, W.J., 2011. Impacts of the cretaceous terrestrial revolution and KPg extinction on mammal diversification. Science 334, 521–524.

Mitchell, K.J., Llamas, B, Soubrier, J, Rawlence, N.J., Worthy, T.H., Wood, J, Lee, M.S.Y., Cooper, A., 2014. Ancient DNA reveals elephant birds and kiwi are sister taxa and clarifies ratite bird evolution. Science 344, 898–900.

Mitchell, K.J., Scanferla, A., Soibelzon, E., Bonini, R., Ochoa, J., Cooper, A., 2016. Ancient DNA from the extinct South American giant glyptodont *Doedicurus sp.* (Xenarthra: Glyptodontidae) reveals that glyptodonts evolved from Eocene armadillos. Mol. Ecol. 25, 3499–3508.

Moore, W.S., 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49, 718–726.

Morgan, C.C., Creevey, C.J., O'Connell, M.J., 2014. Mitochondrial data are not suitable for resolving placental mammal phylogeny. Mammal. Genome 25, 636–647.

Murphy, W.J., Eizirik, E., Johnson, W.E., Zhang, Y.P., Ryder, O.A., O'Brien, S.J., 2001a. Molecular phylogenetics and the origins of placental mammals. Nature 409, 614–618.

Murphy, W.J., Eizirik, E., O'Brien, S.J., Madsen, O., Scally, M., Douady, C.J., Teeling, E., Ryder, O.A., Stanhope, M.J., de Jong, W.W., Springer, M.S., 2001b. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 294, 2348–2351.

Naylor, G., Brown, W., 1997. Structural biology and phylogenetic estimation. Nature 388, 527–528.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2014. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274.

Nilsson, M., Gullberg, A., Spotorno, A.E., Arnason, U., Janke, A., 2003. Radiation of extant marsupials after the K/T boundary: Evidence from complete mitochondrial genomes. J. Mol. Evol 57 (Suppl.), S3–S12.

Nilsson, M.A., Zheng, Y., Kumar, V., Phillips, M.J., Janke, A., 2018. Speciation generates mosaic genomes in kangaroos. Genome Biol. Evol. 10, 33–44.

Nishihara, H., Hasegawa, M., Okada, N., 2006. Pegasoferae, an unexpected mammalian clade revealed by tracking ancient retroposon insertions. Proc. Natl. Acad. Sci. USA 103, 9929–9934.

O'Leary, M.A., Bloch, J.I., Flynn, J.J., Gaudin, T.J., Giallombardo, A., Giannini, N.P., Goldberg, S.L., Kraatz, B.P., Luo, Z.-X., Meng, J., Ni, X., Novacek, M.J., Perini, F.A., Randall, Z.S., Rougier, G.W., Sargis, E.J., Silcox, M.T., Simmons, N.B., Spaulding, M., Velazco, P.M., Weksler, M., Wible, J.R., Cirranello, A.L., 2013. The placental mammal ancestor and the post-K-Pg radiation of placentals. Science 339, 662–667.

Paijmans, J.L.A., Barnett, R., Gilbert, M.T.P., Zepeda-Mendoza, M.L., Reumer, J.W.F., de Vos, J., Zazula, G., Nagel, D., Baryshnikov, G.F., Leonard, J.A., Rohland, N., Westbury, M.V., Barlow, A., Hofreiter, M., 2017. Evolutionary history of saber-toothed cats based on ancient mitogenomics. Current Biology 27, 3330–3336.

Paijmans, J.L.A., Gilbert, M.P.T., Hofreiter, M, 2013. Mitogenomic analyses from ancient DNA. Mol. Phylogenet. Evol. 69, 404–416.

Penny, D., Hasegawa, M., 1997. The platypus put in its place. Nature 387, 549–550.

Pereira, S.L., 2000. Mitochondrial genome organization and vertebrate phylogenetics. Genet. Mol. Biol. 23, 745–752.

Pesole, G., Gissi, C., De Chirico, A., Saccone, C., 1999. Nucleotide Substitution Rate of Mammalian Mitochondrial Genomes. J. Mol. Evol. 48, 427–434.

Philippe, H., Brinkmann, H., Lavrov, D.V., Littlewood, D.T., Manuel, M., Wörheide, G., Baurain, D., 2011. Resolving difficult phylogenetic questions: why more sequences are not enough. PLoS Biol. 9, e1000602.

Philippe, H., Zhou, Y., Brinkmann, H., Rodrigue, N., Delsuc, F., 2005. Heterotachy and long-branch attraction in phylogenetics. BMC Evol. Biol. 5, 50.

Phillips, M.J., Fruciano, C., 2018. The soft explosive model of placental mammal evolution. BMC Evol. Biol. 18, 104.

Phillips, M.J., Gibb, G.C., Crimp, E.A., Penny, D., 2010. Tinamous and moa flock together: mitochondrial genome sequence analysis reveals independent losses of flight among ratites. Syst. Biol. 59, 90–107.

Phillips, M.J., Haouchar, D., Pratt, R.C., Gibb, G.C., Bunce, M., 2013. Inferring kangaroo phylogeny from incongruent nuclear and mitochondrial genes. PloS One 8, e57745.

Phillips, M.J., Lin, Y.-H., Harrison, G., Penny, D., 2001. Mitochondrial genomes of a bandicoot and a brushtail possum confirm the monophyly of australidelphian marsupials. Proc. R. Soc. Lond. B Biol. Sci. 268, 1533–1538.

Phillips, M.J., MacLenachan, P.A., Down, C., Gibb, G.C., Penny, D., 2006. Combined mitochondrial and nuclear protein-coding DNA sequences resolve the Interrelations of the major Australasian marsupial radiations. Syst. Biol. 55, 122–137.

Phillips, M.J., Penny, D., 2003. The root of the mammalian tree inferred from whole mitochondrial genomes. Mol. Phylogenet. Evol. 28, 171–185.

Phillips, M.J., Pratt, R.C., 2008. Family-level relationships among the Australasian marsupial ‘‘herbivores’’ (Diprotodontia: Koala, wombats, kangaroos and possums). Mol. Phylogenet. Evol. 46, 594–605.

Prasad, A.B., Allard, M.W., Green, E.D., 2008. Confirming the phylogeny of mammals by use of large comparative sequence data sets. Mol. Biol. Evol. 25, 1795–1808.

Rambaut, A., 1996. Sequence alignment editor. Available at: http://tree.bio.ed.ac.uk/software/seal.

Rambaut, A., Drummond, A.J., 2007. Tracer v1.6. Available from http://beast.bio.ed.ac.uk/.

Reyes, A., Gissi, C., Catzeflis, F., Nevo, E., Pesole, G., Saccone, C., 2004. Congruent mammalian trees from mitochondrial and nuclear genes using Bayesian methods. Mol. Biol. Evol. 21, 397–403.

Robins, J.H., McLenachan, P.A., Phillips, M.J., McComish, B.J., Matisoo-Smith, E., Ross, H.A., 2010. Evolutionary relationships and divergence times among the native rats of Australia. BMC Evol. Biol. 10, 375 375.

Romiguier, J., Ranwez, V., Delsuc, F., Galtier, N., Douzery, E.J.P., 2013. Less is more in mammalian phylogenomics: AT-rich genes minimize tree conflicts and unravel the root of placental mammals. Mol. Biol. Evol. 30, 2134–2144.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Ropiquet, A., Hassanin, A., 2006. Hybrid origin of the Pliocene ancestor of wild goats. Mol. Phylogenet. Evol. 41, 395–404.

Rose, G.D., Wolfenden, R., 1993. Hydrogen bonding, hydrophobicity, packing, and protein folding. Annu. Rev. Biophys. Biomolecul. Struct. 22, 381–415.

Rubinoff, D., Holland, B.S., 2005. Between two extremes: mitochondrial DNA is neither the Panacea nor the Nemesis of phylogenetic and taxonomic inference. Syst. Biol. 54, 952–961.

Saccone, C., Gissi, C., Lanave, C., Larizza, A., Pesole, G., Reyes, A., 2000. Evolution of the mitochondrial genetic system: an overview. Gene 261, 153–159.

Schmitz, J., Noll, A., Raabe, C., Curakov, G., Voss, R., Kiefmann, M., Rozhdestvensky, T., Brosius, J., Baertsch, R., Clawson, R., Roos, C., Zimin, A., Minx, P., Montague, M.J., Wilson, R.K., Warren, W.C., 2016. Genome sequence of the basal haplorrhine primate *Tarsius syrichta* reveals unusual insertions. Nat. Commun. 7, 12997.

Song, S., Liu, L., Edwards, S.V., Wu, S., 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. Proc. Natl. Acad. Sci. USA 109, 14942–14947.

Springer, M.S., Cleven, G.C., Madsen, O., de Jong, W.W., Waddell, V.G., Amrine, H.M., Stanhope, M.J., 1997. Endemic African mammals shake the phylogenetic tree. Nature 388, 61–64.

Springer, M.S., DeBry, R.W., Douady, C., Amrine, H.M., Madsen, O., De Jong, W.W., Stanhope, M.J., 2001. Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. Mol. Biol. Evol. 18, 132–143.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony* (*and other methods) v4.0b10. Sinauer Associates, Sunderland.

Tilak, M.-K., Justy, F., Debiais-Thibaud, M., Botero-Castro, F., Delsuc, F., Douzery, E.J., 2015. A cost-effective straightforward protocol for shotgun Illumina libraries designed to assemble complete mitogenomes from non-model species. Conserv. Genet. Resour. 7, 37–40.

Waddell, P.J., Cao, Y., Hauf, J., Hasegawa, M., 1999. Using novel phylogenetic methods to evaluate mammalian mtDNA, including amino acid-invariant sites-LogDet plus site stripping, to detect internal conflicts in the data, with special reference to the positions of hedgehog, armadillo and elephant. Syst. Biol. 48, 31–53.

Waddell, P.J., Kishino, H., Ota, R., 2001. A phylogenetic foundation for comparative mammalian genomics. Genome Informat. 12, 141–154.

Welker, F, Collins, M, Thomas, J, et al., 2015. Ancient proteins resolve the evolutionary history of Darwin's South American ungulates. Nature 522, 81–84.

Westbury, M., Baleka, S., Barlow, A., Hartmann, S., Paijmans, J.L.A., Kramarz, A., Forasiepi, A.M., Bond, M., Gelfo, J.N., Reguero, M.A., Lopez-Mendoza, P., Taglioretti, M., Scaglia, F., Rinderknecht, A., Jones, W., Mena, F., Billet, G., de Muizon, C., Aguilar, J.L., MacPhee, R.D.E., Hofreiter, M., 2017. A mitogenomic timetree for Darwin's enigmatic South American mammal *Macrauchenia patachonica*. Nat. Commun. 8, 15951.

Wible, J.R., Wang, Y., Li, C., Dawson, M.R., 2005. Cranial anatomy and relationships of a new ctenodactyloid (Mammalia, Rodentia) from the early eocene of Hubei Province. China. Ann. Carnegie Mus. 74, 91–150.

Woese, C.R., Achenbach, L., Rouviere, P., Mandelco, L., 1991. Archaeal phylogeny: reexamination of the phylogenetic position of *Archaeoglohus fulgidus* in light of

certain composition-induced artifacts. Systematic and Applied Microbiology 14, 364–371.
Wu, J., Hasegawa, M., Zhong, Y., Yonezawa, T., 2014. Importance of synonymous substitutions under dense taxon sampling and appropriate modeling in reconstructing the mitogenomic tree of Eutheria. Genes Genet. Syst. 89, 237–251.
Xu, X., Janke, A., Arnason, U., 1996. The complete mitochondrial DNA sequence of the greater Indian rhinoceros, *Rhinoceros unicornis*, and the phylogenetic relationship among Carnivora, Perissodactyla, and Artiodactyla (+ Cetacea). Mol. Biol. Evol. 13, 1167–1173.
Zink, R.M., Barrowclough, G.F., 2008. Mitochondrial DNA under siege in avian phylogeography. Mol. Ecol. 17, 2107–2121.