

Supplementary Materials for

Inactivation of thermogenic UCP1 as a historical contingency in multiple placental mammal clades

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Materials and Methods

Taxon Sampling.

This study included representatives of 141 vertebrates, 133 of which are mammals (1 monotreme, 4 marsupials, 4 xenarthrans, 11 afrotherians, 59 laurasiatherians, and 54 euarchontoglires). Outgroup representatives included 7 fish and 1 amphibian species. Due to the documented loss of uncoupling protein 1 (*ucp1*) in the archosaur lineage (55), birds and reptiles were not included in the dataset. We employed genome mining, polymerase chain reaction (PCR), and hybridization capture to obtain *ucp1*, *ucp2*, *ucp3*, and sarcolipin (*sln*) coding sequences examined in this study. Taxon sampling and GenBank accession numbers are provided in table S1.

Genome mining.

We used key word searches to identify *ucp1*, *ucp2*, *ucp3*, and *sln* mRNA sequences in GenBank. We also performed standard nucleotide blasts (blastn; 56) against whole-genome shotgun contigs using the “discontinuous megablast” setting and human *ucp1*, *ucp2*, *ucp3*, or *sln* coding sequences (accession numbers: NM_021833.4, U82819.1, U84763.1) as queries. Retrieved contigs from the top blast hits for each species were imported into the program Sequencher versions 5.0 (57), and manually annotated by aligning human *ucp* exons against the individual contigs. In cases where putative exon lengths did not match those of the human loci, exon/intron boundary splice sites were identified following the GT-AG rule (58). For fragmentary and highly degraded *ucp1* pseudogenes (e.g., armadillo, pangolin, hyrax, sloth), we generated dot-plots versus *ucp1* mRNA sequences of closely related species using the EMBOSS:dotmatcher feature in Geneious R6.1 (59) to identify exons. Linear comparison figures illustrating coding regions of these genes were prepared using Easyfig 2.1 (60).

The synteny of *ucp1* is highly conserved among vertebrates with *ucp1* located between the upstream *tbc1d9* and downstream *elmod2* loci (13, 55). Thus, wherever possible, exons of these flanking genes were annotated on *ucp1*-containing contigs to ensure our annotated genes are *ucp1* orthologs.

Finally, discontinuous megablasts against the NCBI sequence read archive (SRA) database were performed for the woolly mammoth (*Mammuthus primigenius*), donkey (*Equus asinus*), fin whale (*Balaenoptera acutorostrata*), and snow leopard (*Panthera uncia*) using queries of known *ucp* coding sequences from the nearest phylogenetic relative (listed below). Retrieved SRAs were imported into Sequencher and assembled against respective reference *ucp* contigs of the African elephant (*Loxodonta africana*), horse (*Equus caballus*), minke whale (*Balaenoptera acutorostrata*), and tiger (*Panthera tigris altaica*) to generate consensus *ucp1*, *ucp2*, *ucp3*, and *sln* coding sequences.

DNA amplification and Sanger sequencing.

The African elephant *ucp1* sequence on GenBank exhibited a 1 bp frameshift deletion in exon 3 (incorrectly annotated on Ensembl). We thus verified this deletion in both African and Asian elephant (*Elephas maximus*) DNA samples via PCR and Sanger sequencing techniques. PCR was also used to obtain *ucp1* sequences from the three-toed brown-throated sloth (*Bradypus variegatus*), silky anteater (*Cyclopes didactylus*), bowhead whale (*Balaena mysticetus*), Grant’s zebra (*Equus quagga boehmi*), black rhinoceros (*Diceros bicornis*), and Indian rhinoceros (*Rhinoceros unicornis*). Specimen information is provided in table S6 (61-64).

Genomic DNA extractions were performed using the DNeasy Blood and Tissue Kit (Qiagen). For the bowhead whale sample, a whole genome amplification was performed using a REPLI-g Mini Kit (Qiagen) following the manufacturer's directions prior to PCR amplification to increase the amount of available DNA. Primers were designed to target each of the six individual *ucp1* exons from the available draft genome of the nearest phylogenetic relative using the Primer Premier 5.0 software (65). PCRs were performed using 2 units of OneTaq DNA polymerase (New England Biolabs) in 20 μ L reactions using the following thermal cycling profile: initial denaturation at 94°C for 2 min 30 sec, followed by 35 cycles at 94°C (denaturation) for 30 sec, 48-62°C (annealing) for 30 sec and 68°C (extension) for 30 sec, followed by a final extension of 5 min at 68°C. Products were visualized on 1.5% agarose gels. In cases where this protocol did not result in a distinct amplification product, nested or hemi-nested PCRs were performed using aliquots of the initial amplification reactions as template DNA. Distinct bands of appropriate size were excised from agarose gels and purified using a GeneJet Gel Extraction Kit (Fermentas). The purified PCR products were either sequenced directly or cloned into pDRIVE cloning vectors (Qiagen) and transformed into *Escherichia coli* plasmids (New England Biolabs). Clones were grown overnight on agar plates at 37°C. Positive clones were identified using a blue-white screening technique, followed by PCR amplification and visualization of the targeted DNA by gel electrophoresis. Cells containing inserts of the correct size were grown overnight in Luria broth culture medium and the DNA was subsequently purified using the Zippy Plasmid Miniprep Kit (Zymo Research). Sequencing reactions were conducted using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) as per the manufacturer's directions. Sequencing reactions were purified using the Zymo Research DNA Sequencing Clean-up Kit (Zymo Research) and sequenced in both directions with an Applied Biosystems 3130 Genetic Analyzer.

DNA hybridization capture and next-generation sequencing.

Methods for obtaining *ucp1*, *ucp2*, *ucp3*, and *sln* sequences from the dugong (*Dugong dugon*) and recently extinct Steller's sea cow (*Hydrodamalis gigas*) are detailed elsewhere (62, 63). Briefly, barcoded DNA libraries suitable for Illumina sequencing were prepared from dugong and Steller's sea cow DNA extracts and hybridized to Agilent SureSelect Capture arrays imprinted with the coding region (plus 25-30 bp of flanking sequence for each exon) of African elephant *ucp1*, *ucp2*, *ucp3*, and *sln* sequences. Eluted DNA fragments were sequenced on Illumina GAIIx and HiSeq2500 genome analyzers, trimmed of adapter sequences, and those <20 bp removed from the dataset (63). Remaining reads were assembled to manatee reference sequences using Geneious. The Steller's sea cow assemblies were manually examined for C→U[T] and G→A DNA damage artifacts (66), with any polymorphic C/T or G/A sites subsequently scored as C or G; non-polymorphic C→T or G→A changes relative to dugong or manatee sequences were treated as genuine (63).

For pygmy hippopotamus (*Choeropsis liberiensis*) and beluga (*Delphinapterus leucas*), genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen). Three μ g of genomic DNA was sheared into fragments of highest concentration at ~180 to 190 bp using a Bioruptor (Diagenode), followed by treatment with PreCR Repair Mix (New England Biolabs). The SureSelect^{XT} Target Enrichment System for Illumina Paired-End Sequencing Library kit (Agilent) was used for library construction and target enrichment. Target enrichment was done with a custom-designed SureSelect biotinylated RNA library that included cow *ucp1*, *ucp2*, and *ucp3* sequences. Target enriched libraries were paired-end 2x100 sequenced on an Illumina

HiSeq 2500 platform at the UC Riverside Institute for Integrative Genome Biology Genomics Core. Per base quality distributions of de-multiplexed fastq files were visualized for both read pair files using FastQC v.0.10.0 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) with the no group setting. Based on these results, FASTX-Toolkit v.0.0.13.2 (http://hannonlab.cshl.edu/fastx_toolkit/index.html) was used to trim the first 3 bases and the last base, which resulted in 97 bp reads. Reads with all but three identical bases or a quality score below 30 at any base position were then filtered out. PRINSEQ lite v.0.20.4 (67) was then used to find read pairs that passed the filtering conditions. These read pairs were then interleaved into a single file using the ShuffleFastq script in RACKJ v.0.95 (<http://rackj.sourceforge.net/Scripts/index.html#ShuffleFastq>).

DNA libraries were also constructed for the Malayan tapir (*Tapirus indicus*), black rhinoceros (*Diceros bicornis*), Indian rhinoceros (*Rhinoceros unicornis*), Sumatran rhinoceros (*Dicerorhinus sumatrensis*) and the extinct woolly rhinoceros (*Coelodonta antiquitatis*). Briefly, previously extracted genomic DNA samples of the black and Indian rhinoceroses (see above) were amplified using a REPLI-g Mini Kit (Qiagen) to increase the quantity of available DNA. DNA fragments were then enzymatically sheared using NEBNext dsDNA Fragmentase (New England Biolabs) and libraries were created using NEXTFlex barcoded adaptors and a NEBNext Fast DNA Library Prep Set for Ion Torrent (New England Biolabs) following the manufacturer's protocol. A DNeasy Blood and Tissue Kit (Qiagen) was used to perform extractions from Malayan tapir and Sumatran rhinoceros blood samples, while ancient DNA was extracted from three woolly rhinoceros bone samples in an ancient DNA dedicated laboratory following the methods described by Dabney *et al.* (68). DNA libraries were constructed for these species using a NEBNext DNA Library Prep Master Mix Set for 454 (New England Biolabs). All libraries were then size selected using an E-gel iBase (Invitrogen) and target enriched using biotinylated 120mer MyBaits RNA probes (Mycroarray) designed with a 4x tiling pattern from the draft genome of the white rhinoceros (*Ceratotherium simum*) following the manufacturer's protocol. Enriched DNA libraries were then amplified in 25 μ l PCR cocktails with the following thermocycling profile: initial denaturation 98°C for 30 sec, 14-20 cycles of 98°C for 20 sec (denaturation), 58°C for 30 seconds (annealing), and 72°C for 30 sec (extension) followed by a final extension period of 72°C for 5 min. Libraries were sequenced at the University of Manitoba on an Ion Torrent PGM platform. Sequenced reads were assembled to white rhinoceros *ucp1*, *ucp2*, *ucp3*, and *sln* reference sequences in Geneious R7.1.9 at 20% maximum mismatch to build consensus sequences. Ancient DNA reads from the woolly rhinoceros libraries were examined for C→T and G→A mutations as described above for the Steller's sea cow.

UCP coding sequence alignments.

Translated *ucp* coding sequence files were created for each species and all sequences visually examined for nonsense, insertion or deletion frameshift, start codon, termination codon, and splice site mutations. Multiple nucleotide sequence alignments were constructed for *ucp1*, *ucp2*, and *ucp3* datasets, both individually and combined, using the program MUSCLE 3.6 (69), and manually adjusted to eliminate ambiguous regions. The single *ucp1* sequence retrieved from the SRA database of the Darwin's ground sloth (*Mylodon darwini*) was deemed to be too short for selection pressure analyses and therefore excluded from the final *ucp1* alignment. Insertions unique to fish (9 bp), marsupials (12 bp), and *Canis lupus familiaris* (6 bp) were also excluded from the *ucp1* alignment. The final *ucp1* alignment included 141 species and totaled 936 bp in length (data file S5). The *ucp2* alignment included 131 species and totaled 948 bp in length (data

file S6), the *ucp3* alignment included 128 species and totaled 936 bp in length (data file S7), while the combined *ucp1-ucp2-ucp3* alignment included 400 loci and totaled 983 bp in length.

UCP gene tree analysis.

To ensure all *ucp* sequences were correctly assigned, a maximum likelihood *ucp1-ucp2-ucp3* tree was constructed with the RAxML 7.2.8 plugin in Geneious using the GTR + Γ option (fig. S4). The *ucp* tree was generated starting with a randomized tree and 500 bootstrap replicates using the “rapid bootstrapping setting and search for the best-scoring ML tree” parameter.

Species tree analysis.

We constructed a species tree for 136 mammals and four outgroups (western clawed frog [*Xenopus tropicalis*], Carolina anole [*Anolis carolinensis*], red junglefowl [*Gallus gallus*], and zebrafinch [*Taeniopygia guttata*]) on the basis of a supermatrix for 30 nuclear (*A2AB*, *ADRB2*, *APP*, *ATP7A*, *Adora3*, *ApoB*, *BCHE*, *BDNF*, *BMII*, *BRCA1*, *BRCA2*, *CHRNA1*, *CMYC*, *CNR1*, *CREM*, *DMP1*, *ENAM*, *EDG1*, *FBN1*, *GHR*, *IRBP*, *MC1R*, *PLCB4*, *PNOC*, *Rag1*, *Rag2*, *SWS1*, *TTN*, *TYR1*, *VWF*) and 11 mitochondrial genes (*12S rRNA*, *16S rRNA*, *CYTB*, *COI*, *COII*, *COIII*, *ND1*, *ND2*, *ND3*, *ND4*, *ND5*). Sequences were obtained from GenBank, Ensembl, PreEnsembl, and other genomic resources (*i.e.*, gigadb.org for *Ursus maritimus* and *Panthalops hodgsonii*, www.bowhead-whale.org for *Balaena mysticetus*) and were culled from larger sequence alignments for all available mammalian species (M.S.S., unpublished). Accession numbers, scaffold numbers, etc., are given in data file S8. Alignment-ambiguous regions were excluded prior to phylogenetic analysis. Additional sites with missing data/gaps for our reduced set of species were removed in RAxML. The final alignment comprises 50,879 bp (data file S9). A species tree was obtained with RAxML-HPC2 on XSEDE (RAxML version 8.1.11) on CIPRES (70). The RAxML analysis was performed with 32 partitions, each of which was given its own GTR + Γ model of sequence evolution. The 32 partitions included one for each of 30 nuclear genes, one for 12S rRNA + 16S rRNA, and one for nine mitochondrial protein-coding genes. We employed the GTRGAMMA model for both rapid bootstrap analysis (500 bootstrap iterations) and a search for the best ML tree.

Timetree analyses.

Timetree analyses were performed on the 41-gene supermatrix tree with the mcmctree program in PAML 4.5 (71), which implements the MCMC algorithms of Rannala and Yang (72). Analyses were performed with the autocorrelated rates model. Each of 32 partitions (see above) was allowed to have its own GTR + Γ model of sequence evolution. We set one time unit = 100 million years (Ma). Analyses were run with cleandata = 0. Shape (α) and scale (β) parameters for the gamma prior of the overall rate parameter μ (*i.e.*, rgene_gamma in mcmctree) were 1 and 4.74, respectively. Calculations for the shape and scale parameters of the gamma prior for the rate-drift parameter (*i.e.*, sigma2_gamma in mcmctree) assumed an age of 344 Ma for the most recent common ancestor of Tetrapoda (average of minimum and maximum constraints in Benton *et al.*; 73). Chains were run for 100,000 generations after a burn-in of 10,000 generations, and were sampled every 20 generations. We employed hard-bounded constraints for 37 nodes including the root node (Tetrapoda). Minimum ages were based on the oldest crown fossils that are assignable to each clade while maximum ages were based on stratigraphic bounding, phylogenetic bracketing, and phylogenetic uncertainty (63, 74-76). Stratigraphic bounds were extended by one stage for younger deposits (late Miocene, Pliocene, Pleistocene) and two stages

for older deposits (middle Miocene and earlier). Minimum and maximum constraints (47, 63, 73, 77-158) are summarized in table S7. Stage boundaries are from the International Chronostratigraphic Chart v 2014/02 (www.stratigraphy.org; 159).

Evolutionary selection pressure and inactivation analyses.

The CODEML program in the PAML 4.8 software package (71) was employed to estimate the nonsynonymous/synonymous substitution rate ratio (dN/dS or ω value) in order to infer the modes of natural selection acting upon the three *ucp* loci. All frameshift insertions in the alignments were deleted, and nonsense mutations were recoded as missing data prior to analysis. Our constructed species tree was used to guide the phylogeny of all CODEML runs with outgroup fish species added to the guide tree following the phylogenetic relationships described by Near *et al.* (160). All CODEML runs were performed using the F3x4 codon frequency model. The free-ratio (M1) model, which calculates an independent ω value for every branch of the tree, was performed for all three *ucp* alignments (data files S5-S7). Results of this analysis for *ucp1* were used as a starting point to target specific lineages later tested with the branch (M2) model for neutral evolution or positive selection. Target lineages for these analyses included the stem placental branch, the stem pinniped branch, the pygmy hippo branch, the camel branch, and finally all pseudogenic *ucp1* branches. Individual M2 branch models for the stem placental, pinniped, pygmy hippo, and camel branches were performed using the same branch categories described below for the *ucp1* pseudogene analysis plus an additional branch category to target the lineage(s) of interest. Likelihood ratio tests (table S2) were then performed against the *ucp1* pseudogene M2 branch model to test if the obtained ω values were significantly different from all functional placental branches. The M2 models were run with the method = 0 setting which calculates all branch lengths simultaneously.

Branch-site analyses were also performed to identify potential amino acids under positive selection that may have contributed to the gain of non-shivering thermogenic function of *ucp1* in a stem placental ancestor. Here, the stem placental branch of the species tree was set to the foreground while all others were background branches. To reduce the possibility of signatures of neutral evolution skewing the results, pseudogenes were not included in this analysis, resulting in a data set of 115 species. The branch-site MA model, under the Bayes empirical Bayes method, resulted in the identification of eight sites with a posterior probability >0.95 of being greater than one (table S2). However, when tested against the null model, where the ω values of positively selected sites under the MA model are fixed at neutrality ($\omega = 1$), the likelihood ratio test demonstrated that omega values for these sites were not significantly greater than one ($2\Delta\ell = 0.706$, $df = 1$, $p = 0.400$).

For each *ucp1* pseudogenization event we calculated the transition point from purifying selection to neutral evolution following the method and equations of Meredith *et al.* (26). The *ucp1* inactivations are considered to have occurred at or following this date. Branches were first divided into three categories: functional, transitional (mixed), and pseudogenic. Functional branches are those that lead to internal or terminal nodes with an intact copy of *ucp1* and presumably evolved under functional constraints. Transitional branches record the first evidence of *ucp1* pseudogenization (*e.g.*, stop codon, frameshift mutation) and are deemed to contain both functional and pseudogenic segments that evolved under purifying selection and neutral evolution, respectively. Pseudogenic branches post-date mixed branches and are expected to have dN/dS values at or near the neutral value of 1 in the absence of purifying or positive selection (26). In taxonomic clades where all members share inactivating *ucp1* mutations (*i.e.*,

Cetacea, Equidae, Proboscidea, Sirenia, Pilosa), the transitional branches were deemed to be immediately ancestral to the radiation of the clade. The guide tree was labeled in order to calculate ω values for all functional non-mammalian vertebrate branches, all functional non-placental mammalian branches, all functional placental mammal branches, and all pseudogenetic placental mammal branches as distinct branch categories as well as each individual transitional branch using the M2 branch model. The equations developed by Meredith *et al.* (26) were employed to estimate transition dates from purifying selection to neutral evolution of *ucp1* along transitional branches using our upper and lower nodes for each branch from our time-calibrated phylogeny (data file S2).

Paleotemperature calculations.

We used stable oxygen isotope ($\delta^{18}\text{O}$) values (‰) of benthic foraminifera (supplementary Fig. 3 dataset of Friedrich *et al.*; 161) as a proxy for global temperature over the last ~82 Ma (see Fig. 3). Within this window, paleotemperatures were calculated (162) from the Cretaceous to Oligocene as: temperature ($^{\circ}\text{C}$) = $16.5 - 4.8(\delta\text{c} - \delta\text{w})$, where δc is the measured $\delta^{18}\text{O}$ value of foraminifera calcite and δw (-1.0 ‰) is the estimated value for an ice-free ocean (163).

Fossil body mass and diversity.

Fossil body mass estimates recorded at each geological stage for Cetacea, Sirenia, Proboscidea, Hyracoidea, and Equidae were assembled from literature sources (38, 44, 45, 164-181) (data file S10). We also downloaded all species occurrence records for these eutherian clades from each geological stage ranging from the Selandian to the Holocene from the Paleobiology Database (www.fossilworks.org) and other literature sources (38, 40, 164, 171, 178, 181-193). Species and genera counts were then plotted for the median date of each stage (data file S10). Owing to fragmentary fossil records and few body mass estimates for early Xenarthra, Pholidota, and Suidae, these groups were not included in these analyses.

Diversification analysis.

We selected five fully resolved timetrees (numbers 1, 101, 201, 301, 401) from Faurby and Svenning (194) to allow for phylogenetic uncertainty. Each of these trees included all extant mammalian species plus late Quaternary extinct mammals. We pruned all non-placental taxa from these trees. For the remaining placentals, we only retained extant taxa and recently extinct taxa that are included in Wilson and Reeder (195). After pruning, our trees comprised 5138 placental species. The presence versus absence of a functional copy of *ucp1* was inferred from the condition of the gene in the closest relative for which there is DNA sequence evidence, supplemented by evidence from shared mutations and our estimates for inactivation times of *ucp1* in UCP1-minus lineages. For example, molecular data provide evidence for inactivation of *ucp1* in representatives of all three clades of Xenarthra (armadillos, anteaters, sloths), and our inactivation date for this gene is estimated in the latest Cretaceous. We therefore coded all xenarthrans as ‘UCP1-minus’. Similarly, diverse rodent and bat taxa with genome sequences all have intact copies of *ucp1*. Based on this evidence, and the general absence of very large rodents and bats, we coded all extant rodent and bat species as ‘UCP1-plus’. Data file S10 contains all of the *ucp1* codings that were employed in our diversification analyses, which were performed with the BiSSE method (49) as implemented in diversitree in R (50). We compared two diversification models, each of which fixed the transition rate from UCP1-minus to UCP1-plus at zero given the absence of empirical evidence for such transitions and the low probability of

resuscitating a functional copy of a gene from an inactivated copy with frameshift and/or premature stop codon mutations. The first model allowed for separate speciation (λ) and extinction (μ) rates in UCP1-plus taxa versus UCP1-minus taxa, whereas the second model constrained equal diversification rates in UCP1-plus taxa and UCP1-minus taxa (*i.e.*, $\lambda_0 = \lambda_1$, $\mu_0 = \mu_1$).

Data Availability: New sequences obtained in this study are archived at GenBank (accession numbers XXX to YYY).

Contributions: M.J.G. conceived the project, designed research, conducted experiments, performed selection and dating analysis, interpreted the results, and prepared the figures; K.L.C conceived the project, designed research, interpreted the results, prepared the figures, and wrote the manuscript; M.J., J.R.T. and M.H. designed research and interpreted the results; J.S., J.L.A.P., N.W., and A.V.S. conducted the hybridization capture and sequencing experiments; M.S.S. performed phylogenetic, timetree, selection, and diversification analysis, interpreted the results, and assisted with manuscript writing; All authors discussed the results and helped revise the manuscript.

Competing financial interests: The authors declare no competing financial interests.

Exon 1

1 10 20 30 40 50
|...|...|...|...|...|...|...|...|...|...|...|
Homo sapiens ATGGGGGGCCTGA-CAGCCTCGGACGTACACCCGACCC--TGGGGGTCC
Bradypus variegatus ???
Choloepus hoffmanni ???
Mylonodon darwini ???
Cyclopes didactylus ???
Dasypus novemcinctus ATGGGGCGCCAGGCCTCCCGCGGGCTCACCCCCGCG-----
Procavia capensis CTG--AGTTAAGA-CAACCTCAGAAATGCCGCCTAC-----GCA
Elephas maximus ACGGTAGGCCAGA-CGACCGCAGACGTGCCCGGACCATGGTGGGGGTCA
Mammuthus primigenius ACGGTAGGCCAGA-CGACCGCAGACGTGCCCGGACCATGGTGGGGGTCA
Loxodonta africana ACCGTAGGCCAGA-CGACCGCAGACGTGCCCGGACCATGGTGGGGGTCA
Trichechus manatus ATGGTGGGCCAGA-CTACCTGGGATGTGCCCCGACCA---TGGGCGTCA
Dugong dugon ATGGTGGGCCAGA-CTACCTCGGATGTGCCCCGACCA---TGGGCGTCA
Hydrodamalis gigas ATGGTGGGCCAGA-CTACCTCGGATGTGCCCCGACCA---TGGGCGTCA
Sus scrofa CTGTCAGGA-TGA-CAGTTCCTGAAGTGCCCCGACCA---TAGCGGTCA
Sus verrucosus CTGTCAGGA-TGA-CAGTTCCTGAAGTGCCCTCGACCA---TAGCGGTCA
Sus cebifrons CTGTCAGGA-TGA-CAGTTCCTGAAGTGCCCTCGACCA---TAGCGGTCA
Physeter macrocephalus ATGGTGGGACTCG-CAGCCTCATACTGCCCCGACCA---TGGGCGTCA
Delphinapterus leucas -----
Lipotes vexillifer ATGGTGGGACTCG-CAGCCTCAGACGTGCCCCGACCA---TGGGCGTCA
Balaena mysticetus ATGGTGGCACTCA-CAGCCTCAGACGTGCCCCGACCA---TGGGCGTCA
Balaenoptera acutorostrata ATGCTGGGACTCA-CAGCCTCAGACGCGCCCCGACCG---TGGGCGTCA
Balaenoptera physalus ATGCTGGGACTCA-CAGCCTCAGACGCGCCCCGACCA---TGGGCGTCA
Balaenoptera bonaerensis ATGCTGGGACTCA-CAGCCTCAGACGCGCCCCGACCA---TGGGCGTCA
Equus asinus ATGGTGGGGCCCA-CAGCCTCGGACCCGCCCCGACCA---TGGGCGTCA
Equus quagga boehmi ATGGTGGGGCCCA-CAGCCTCGGACCGCCCCGACCA---TGGGCGTCA
Equus przewalskii ???
Equus caballus ATGGTGGGGCCCA-CAGCCTCGGACGTGCCCCGACCA---TGGGCGTCA
Manis pentadactyla -----

51 60 70 80 90 100
|...|...|...|...|...|...|...|...|...|...|...|
Homo sapiens AGCTCTTCTCAGCTGGA--ATAGCGGCGTGCTTGGCGGACGTGATCACC
Bradypus variegatus ???
Choloepus hoffmanni ???
Mylonodon darwini ???
Cyclopes didactylus ???
Dasypus novemcinctus -----GCGGAAACGAGGCGGC-----GGCAGGTGCACCCCGC
Procavia capensis ACCTCTTCTCAGCTGGA--GTGGCGGCCTGCTTGGCCGGTGTGATCACC
Elephas maximus AGATCTTCTCAGCGGA--GTGGCGGCCTGCTTGGCAGATGTAATTACC
Mammuthus primigenius AGATCTTCTCAGCGGA--GTGGCGGCCTGCTTGGCAGATGTAATTACC
Loxodonta africana AGATCTTCTCAGCGGA--GTGGCGGCCTGCTTGGCAGATGTAATTACC
Trichechus manatus AGATCGTCTCAGCTGGA--GTGTGGCCTGTTTGGCGGATGTGATCACC
Dugong dugon AGATTGTCTCAGCTGGA--GTGTGGCCTGTTTGGCGGATGTGATCACC
Hydrodamalis gigas AGATTGTCTCAGCTGGA--GTGTGGCCTGTTTGGCGGATGTGATCACC
Sus scrofa AGATCTTCTCAGCTGGA--GCAGCGCCTGCGTGGCAGATGTGATCACC
Sus verrucosus AGATCTTCTCAGCTGGA--GCGGCGCCTGCGTGGCAGATGTGATCACC
Sus cebifrons AGATCTTCTCAGCTGGA--GCGGCGCCTGCGTGGCAGATGTGATCACC
Physeter macrocephalus AGATCTTCTCGGCTGGA--GTGGCGGCCTGCGTGGCGGATGTGATCACC
Delphinapterus leucas -----
Lipotes vexillifer AGATCTTCTCGGCTGGA--GTGGCGGCCAGCGTGGCGGATGTGATCACC
Balaena mysticetus CGATCTTCTCGGCTGGA--GTGGCGGCCGCGCTGGCGGATGTGATCACC
Balaenoptera acutorostrata CGATCTTCTCGGCTGGA--GTGGCGGCCGCGCTGGCGGATGTGATCACC
Balaenoptera physalus CGATCTTCTCGGCTGGA--GTGGCGGCCGCGCTGGCGGATGTGATCACC
Balaenoptera bonaerensis CGATCTTCTCGGCTGGA--GTGGCGGCCGCGCTGGCGGATGTGATCACC
Equus asinus AGATCTTCTCGGCTGGA--GTGGCGGCCTGCGTGGCGGACGTGATCGCC
Equus quagga boehmi AGATCTTCTCGGCTGGA--GTGGCGGCCTGCGTGGCGGACGTGATCGCC

Equus przewalskii
Equus caballus
Manis pentadactyla

??CCTGCGTGGCGGACGTGATCGCC
AGATCTTCTCGGCTGGA--GTGGCGCCTGCGTGGCGGACGTGATCGCC

Homo sapiens
Bradypus variegatus
Choloepus hoffmanni
Mylodon darwinii
Cyclopes didactylus
Dasybus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon
Hydrodamalis gigas
Sus scrofa
Sus verrucosus
Sus cebifrons
Physeter macrocephalus
Delphinapterus leucas
Lipotes vexillifer
Balaena mysticetus
Balaenoptera acutorostrata
Balaenoptera physalus
Balaenoptera bonaerensis
Equus asinus
Equus quagga boehmi
Equus przewalskii
Equus caballus
Manis pentadactyla

101 110 120 130
|...|...|. ...|. ...|. ...|. ...|. ...|
TTCCCGC--TGGACACGGCCAAAGTCCGGCTCCAGgt
??
??
??
??
CTCCTCCGCTGGG-GCCGCCAGCCTGGGGCTGCAGct
TTCCAC--TGGACACTGCCAAAGTTCGGGTACAGgc
TTCCCGC--TGATCGCGGCCAAAGTTCGGCTGCAGat
TTCCCGC--TGGTCGCGGCCAAAGTTCGGCTGCAGat
TTCCAC--TGGACACGGCCAAGGTTTCGGCTACAGgt
TTCCAC--TGGACACGGCCAAGGTTTCGGCTACAGgt
TTCCAC--TGGACACGGCCAAGGTTTCGGCTACAGgt
TTCCAC--TGGACACGGCCAAGGTTTCGGCTACAGgt
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TTTGACCTTGGACACCGCCAAAGTCCGGCTACAGgt
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TTCCCGC--TGGACACGGCCAAGTCCGGCTACAGgg
TTCCCGC--TGGACACGGCCAAGTCCGGCTACAGgt
TTCCCGC--TGGACACGGCCATAGTCCGGCTACAGgt
TTCCCGC--TGGACACGGCCAAGTCCGGCTACAGgt
TTCCCGC--TGGACACGGCCATAGTCCGGCTACAGgt
TTCCCGC--TGGACACGGCCAAGTCCGGCTACAGgt
TTCCCGC--TGGACACGGCCAAGTCCGGCTACAGgt
TTCCCGC--TGGACACGGCCAAGTCCGGCTACAGgt
TTCCCGC--TGGACACGGCCAAGTCCGGCTACAGgt

Exon 2

Homo sapiens
Bradypus variegatus
Choloepus hoffmanni
Mylodon darwinii
Cyclopes didactylus
Dasybus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon
Hydrodamalis gigas
Sus scrofa
Sus verrucosus
Sus cebifrons
Physeter macrocephalus
Delphinapterus leucas
Lipotes vexillifer
Balaena mysticetus
Balaenoptera acutorostrata

136 140 150 160 170 180
|...|...|. ...|. ...|. ...|. ...|. ...|
agGTCCAAGGTGAATGCCGACGTCCAGTGTTATTAGGTATAAAAGGTGTCCTG
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agATCCAAGGTGAAGGTGAGAAATCCCATGCTATTAAGTACAAAGGTCTCCTG
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agATCCAAGGTGAATGCCAGACCTCCAGTGCCATTAGGTATAAAAGGTGTCTTG
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agGTCCAATGCGAATGCCAGACCTCCAGTGCTGTTAGGTATAAAAGGTGTCTTG

Balaenoptera physalus
Balaenoptera bonaerensis
Equus asinus
Equus quagga boehmi
Equus przewalskii
Equus caballus
Manis pentadactyla

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agATCCAAGGCGAGCGCCAGACCTCCAGTGCCCTGAGGTATAACGGTATCCTG
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Homo sapiens
Bradypus variegatus
Choloepus hoffmanni
Myiodon darwinii
Cyclopes didactylus
Dasyopus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon
Hydrodamalis gigas
Sus scrofa
Sus verrucosus
Sus cebifrons
Physeter microcephalus
Delphinapterus leucas
Lipotes vexillifer
Balaena mysticetus
Balaenoptera acutorostrata
Balaenoptera physalus
Balaenoptera bonaerensis
Equus asinus
Equus quagga boehmi
Equus przewalskii
Equus caballus
Manis pentadactyla

186 190 200 210 220 230
|...|...|...|...|...|...|...|...|...|...|...|...|
GGAACAATCACCCTGTGGTAAAAACAGAAGGGCGGATGAAACTCTACAG
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GGAACAGTGCACACTGGCAAAAACGGAAGGGCCAATGAA--CCTACCG
GAAACAACCACCCTTGACAAAAACAGAGGGGCAATGAAACTATAGCT
GGAACAATCACTACTCTGGCAAAAACGGAAGAGCCAATGAAACTCTATAG
GGAACAATCACTACTCTGGCAAAAACGGAAGAGCCAATGAAACTCTATAG
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GGAACAATCACCCTCTGGCAAAAACAGAAGGGCCAATGAAACTCTACAG

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GGAACAATCACCCTCTGGCAAAAACAGAAGGGCCAATGAAACTCTACAG
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GGGACAATCACCCTCTGGCAAAAACAGAAGGGCCAATGAAACTCTACAG
GGGACAATCACCCTCTGGCAAAAACAGAAGGGCCAATGAAACTCTACAG
GGGACAATCACCCTCTGGCAAAAACAGAAGGGCCAATGAAACTCTACAG

Homo sapiens
Bradypus variegatus
Choloepus hoffmanni
Myiodon darwinii
Cyclopes didactylus
Dasyopus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon
Hydrodamalis gigas
Sus scrofa
Sus verrucosus
Sus cebifrons
Physeter microcephalus
Delphinapterus leucas
Lipotes vexillifer

236 240 250 260 270 280
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??
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--GCTGCCTGCTGGCCTTCAGAGACAAATAAGCTTTGCTCTCTTAGAA
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TGGGCTGCCTGCTGGCCTTCAGAGACAAATAAGCTTTGCTCTCTTAGGA
TGGGCTGCCTGCTGGCCTTCAGAGACAAATAAGCTTTGCTCTCTTAGGA
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TGGGCTGCCAGCTGGCCTCCAGAGACAAATAAGCTTCTGCTCTCTTAGGA

Balaena mysticetus
Balaenoptera acutorostrata
Balaenoptera physalus
Balaenoptera bonaerensis
Equus asinus
Equus quagga boehmi
Equus przewalskii
Equus caballus
Manis pentadactyla

CGGGCTGCCTGCTGGCCTCCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
CGGGCTGCCTGCTGGCCCCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
TGGGCTGCCAGCTGGCCTCCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
CGGGCTGCCTGCTGGCCTCCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
CGGGCTGCCTGCTGGCCTCCAGAGATAAATAAGCTTCGCTCTCTGAGGA
CGGGCTGCCTGCTGGCCTCCAGAGATAAATAAGCTTCGCTCTCTGAGGA
CGGGCCGCCTGCTGGCCTCCAGAGATAAATAAGCTTCGCTCTCTGAGGA
CGGGCCGCCTGCTGGCCTCCAGAGATAAATAAGCTTCGCTCTCTGAGGA

Homo sapiens
Bradypus variegatus
Choloepus hoffmanni
Myiodon darwinii
Cyclopes didactylus
Dasypus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon
Hydrodamalis gigas
Sus scrofa
Sus verrucosus
Sus cebifrons
Physeter macrocephalus
Delphinapterus leucas
Lipotes vexillifer
Balaena mysticetus
Balaenoptera acutorostrata
Balaenoptera physalus
Balaenoptera bonaerensis
Equus asinus
Equus quagga boehmi
Equus przewalskii
Equus caballus
Manis pentadactyla

286 290 300 310 320 330
|...|...|...|...|...|...|...|...|...|...|...
TCGGCCTCTACGACACGGTCCAGGAGTTCCCTCACCGCAGGGAAAGAAAgT
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TGGGACTCTAGGATACCGTCCCGCGGC-TTTCACCGCAGGGAAACCAGca
TTGGCTTCTGTGACA-GGTTTCAGGAGTACTTCACCTGGAGGAAAGAAAgT
CTGGCCTCTATGATATGGCCCAGGAGTATTCCACTGCAGAGAAAGAAAgT
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TTGGCCTCTATGATACTGTCCAGGAGTACTTCACCTGCAGGGFAAGAAAgT
TTGGCCTCTATGATACTGTAGGAGTACTTCACCTGCAGGGFAAGAAAgT
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TCACCCTCTATGATACCATCCAGG-----GAGAGAAAgT
TCAGCCTCTATGATACCATCCAGG-----GAGAGAAAgT
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TTGGCCTCTATGATACGGTCCAGGAGTTCTTCACCACAGGGAAAGAAAgT

Exon 3

Homo sapiens
Bradypus variegatus
Choloepus hoffmanni
Myiodon darwinii
Cyclopes didactylus
Dasypus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon
Hydrodamalis gigas
Sus scrofa
Sus verrucosus

334 340 350 360 370 380
|...|...|...|...|...|...|...|...|...|...|...|...
agCAGCACCTAGTTT TAGGAAGCAAGATTTTAGCTGGTCTAACGACTGGAGGAGT
??
??
ag-----GTAGTTTAGAAATCAAGATTTTTCAGTTGGCTTAACAACCTGGAGAAGT
??

aaCTCCAGCTAGTTT TAGGAAGCAAGATCGCTGCTGGCTTAAGGACTGGAGGAGT
aaCTCCAGCTAGTTT TAGGAAGCAAGATCGCTGCTGGCTTAAGGACTGGAGGAGT
aaCTCCAGCTAGTTT TAGGAAGCAAGATCGCTGCTGGCTTAAGGACTGGAGGAGT
agCACCGGCTAGTTT TAGGAAGCAAGATCTCTGCTGGCTTAACGACTGGAGGAGT
agCACCGGCTAGTTT TAGGAAGCAAGATCTCCGCTGGCTTAACGACTGGAGGAGT
agCACCGGCTAGTTT TAGGAAGCAAGATCTCCGCTGGCTTAATGACTGGAGGAGT

Sus cebifrons
Physeter macrocephalus
Delphinapterus leucas
Lipotes vexillifer
Balaena mysticetus
Balaenoptera acutorostrata
Balaenoptera physalus
Balaenoptera bonaerensis
Equus asinus
Equus quagga boehmi
Equus przewalskii
Equus caballus
Manis pentadactyla

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aa caac ag CTAGTTT TAGGAAGCCAGGTCTCAGCGGGCCTAACGACTGGCGGTGT
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aa caac gg CTAGTTT TAGTGAAGAAGCAGGGTCTCAGCGGGCCTAACGACTGGCGG
aa caac gg CTAGTTT TAGGAAGCAGGGTCTCAGCGGGCCTAACGAATGGCGGCGT
aa caac gg CTAGTTT TAGGAAGCAGGGTCTCAGCGGGCCTAACGAATGGCGGCGT
aa caac gg CTAGTTT TAGGAAGCAGGGTCTCAGCGGGCCTAACGACTGGCGGCGT
ag-----CTAGTTT TAGGAAGCAAGGTCTCAGCTGGCCCTAACGACTGGAGGAGT
ag-----CTAGTTT TAGGAAGCAAGGTCTCAGCTGGCCCTAACGACTGGAGGAGT
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ag-----CTAGTTT TAGGAAGCAAGGTCTCAGCTGGCCCTAACGACTGGAGGAGT
agCAACTTTCAGTTAGGAAGTAAGATCTCAGCTGAGCTAA GACTAAAGGAGA

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385 390 400 410 420 430

Homo sapiens
Bradypus variegatus
Choloepus hoffmanni
Mylodon darwini
Cyclopes didactylus
Dasypus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon
Hydrodamalis gigas
Sus scrofa
Sus verrucosus
Sus cebifrons
Physeter microcephalus
Delphinapterus leucas
Lipotes vexillifer
Balaena mysticetus
Balaenoptera acutorostrata
Balaenoptera physalus
Balaenoptera bonaerensis
Equus asinus
Equus quagga boehmi
Equus przewalskii
Equus caballus
Manis pentadactyla

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|...|...|...|...|...|...|...|...|...|...|
GGCAGTATTTCATTGGGGCAACCCACAGAGGTCGTGAAAGTCAGACTTCAAG
????????????????????????????????????????????????????????
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GGCCAT????????????????????????????????????????????????????
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GGCAGTGGTTCACT GGCAACCCACAGAGGTTCGTGGAGGCTAGACTTCAAG
GGCAGTGGTTCACT GGCAACCCACAGAGGTTCGTGGAGGCTAGACTTCAAG
GGCAGTGGTTCACT GGCAACCCACAGAGGTTCGTGGAGGCTAGACTTCAAG
GGCGGTATTTCATTGGGGCAACCCACAGAGGTTCGTGCAGGTTCAGACATCAAG
GGTGGTATTTCATTGGGGCAACCCACAGAGGTTCATGCAGGTTCAGACATCAAG
GGCGGTATTTGCTGGGGCAACCCACAGAGGTTCATGCAGGTTCAGACATCAAG
-----
GGCCGTGTTCATTGGGGCAACGCACAGAGGTAGTGAAGGTTCAGACTTCAAG
-----TGGTGAAGGTTCAGACTTCAAG
GGCCGTGTTCGTTGGGGCAACCCACAGAGGTAGTGATGGTTCAGACTTCAAG
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GGCTGTGTTCATTGGGGCAACCCACAGAGGTAGTGAAGGTTCAGACTTCAAG
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GGCAGTATTTCATTGGGGCAACCCACAGAGGTTCGTGAAGGTTCAGACTTCAAG
GGCAGTATTTCATTGGGGCAACCCACCGAGGTTCGTGAAGGTTCAGACTTCAAG
GACAGTATTTATTGGGCAAC CACAGAGGTTGTGAAGGTTCAGACTTCAAG

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436 440 450 460 470 480

Homo sapiens
Bradypus variegatus
Choloepus hoffmanni
Mylodon darwini
Cyclopes didactylus
Dasypus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon

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|...|...|...|...|...|...|...|...|...|...|
CACAGAGCCATCTCCACGGAATCAAACCTCGCTACACGGGACTTATAAT
????????????????????????????????????????????????????????
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????????????????????????????????????????????????????????
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CACAGAGCCATCTACATGGTCTCAAACCGTGCTACTGGGACTTACAAT
CACAGAGCCATCTACATGGTCTCAAACCGTGCTACTGGGACTTACAAT
TGCAGAGCCATCTATGTGGTCTCAAACCGTGCTACTGGGACTTACAAT
TGCAGAGCCATCTGTGTGGTCTCAAACCGTGCTACTGGGACTTACAAT

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Hydrodamalis gigas TGCAGAGCCATCTGTGTGGTCTCAAACCGCGCTACACTGGGACTTACAAT
Sus scrofa -----
Sus verrucosus -----
Sus cebifrons -----
Physeter microcephalus CGCCGAGCCGTCTACGTGGTCCCAAACCTCGCCACGCTGGGACTTACAAT
Delphinapterus leucas CGCAGAGCCATCTGCATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Lipotes vexillifer CGCTGAGCCATCTACGTGGTCCCAAACCTCACTACACTGGGACTTACGAT
Balaena mysticetus TGCCAAGCGGTCTACATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Balaenoptera acutorostrata CGCCAAGCAGTCTACATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Balaenoptera physalus CGCCAAGCSGTCTACATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Balaenoptera bonaerensis CGCCAAGCAGTCTACATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Equus asinus CGCAGAGCCATCTGCATGGTCCCAAACCTCGCTACACTGGGACTTATAAT
Equus quagga boehmi CGCAGAGCCATCTGCATGGTCCCAAACCTCGCTRCACACTGGGACTTATAAT
Equus przewalskii CGCAGAGCCATCTGCATGGTCCCAAACCGCGCTACACTGGTACTTATAAT
Equus caballus CGCAGAGCCATCTGCATGGTCCCAAACCGCGCTACACTGGTACTTATAAT
Manis pentadactyla CACAGACCCATCCACATGGTGTCAAACCTCACTACATGGGGACTTAC-T

486 490 500 510 520 530
|...|...|...|...|...|...|...|...|...|...|...
GCGTACAGAATAATAGCAACAACCGAAGGCTTGACGGGTCTTTGGAAAGgt
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Homo sapiens GCGTACAGAATAATAGCAACAACCGAAGGCTTGACGGGTCTTTGGAAAGgt
Bradypus variegatus ???
Choloepus hoffmanni ???
Mylonodon darwini ???
Cyclopes didactylus ???
Dasypus novemcinctus -----
Procyon capensis -----
Elephas maximus GCTGACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGGAAAGgt
Mammuthus primigenius GCTGACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGGAAAGgt
Loxodonta africana GCTGACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGGAAAGgt
Trichechus manatus GCTTACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGAAAGgt
Dugong dugon GCTTACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGAAAGgt
Hydrodamalis gigas GCTTACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGAAAGgt
Sus scrofa -----
Sus verrucosus -----
Sus cebifrons -----
Physeter microcephalus GCTCACAGAATTATAGCAACAACAGAAGGCTTGATGGGGCTTTGGAAAGat
Delphinapterus leucas GCTTACAGAATTATAGCAACAACAGAAGGCTTGACGGGCTTTGGAAAGgt
Lipotes vexillifer GCTCACAGAATTATAGCAACAACAGAAAGCTTGACGGGGCTTTGGAAAGgt
Balaena mysticetus GCTTATAGAATTATAGCAACAACAGAAGGCTTGACGGGGCTTTGGAAAGgt
Balaenoptera acutorostrata GCTTACAGAATTATAGCAACAACAGAAGGCTTGATGGGGCTTTGGAAAGgt
Balaenoptera physalus GCTTACAGAATTATAGCAACAACAGAAGGCTTGACGGGGCTTTGGAAAGgt
Balaenoptera bonaerensis GCTTACAGAATTATAGCAACAACAGAAGGCTTGATGGGGCTTTGGAAAGgt
Equus asinus GCCTACAGAATTACAGCAACAACAGAAGGCTGGACGGGTCTTTGGAAAGgt
Equus quagga boehmi GCCTACAGAATTACAGCAACAACAGAAGGCTGGACGGGTCTTTGGAAAGgt
Equus przewalskii GCCTACAGAATTACAGCAACAACAGAAGGCTGGACGGGTCTTTGGAAAGgt
Equus caballus GCCTACAGAATTACAGCAACAACAGAAGGCTGGACGGGTCTTTGGAAAGgt
Manis pentadactyla GCTGACAGAATTAGAGCAACAACAGGCTTGACAGATCTTTGGAAAGct

Exon 4

535 540 550 560 570 580
|...|...|...|...|...|...|...|...|...|...|...|...
Homo sapiens agGGACTACTCCCAATCTGATGAGAAGTGTTCATCATCAATTGTACAGAGCTAG
Bradypus variegatus ???
Choloepus hoffmanni ???
Mylonodon darwini ???
Cyclopes didactylus ???
Dasypus novemcinctus -----
Procyon capensis -----
Elephas maximus agGGACTACTCTCAGTCTGACAAGAAATATCATCATCAGTTGTACAGAGCTAG

Mammuthus primigenius agGGACTACTCTCAGTCTGACAAGAAATATCATCATCAGTTGTACAGAGCTAG
Loxodonta africana agGGACTACTCTCAATCTGACAAGAAATATCATCATCAGTTGTACAGAGCTAG
Trichechus manatus agGGATTACTCCCAATCTGACAAGAAATGTCATCATCAATTTGTACAGAGCTAG
Dugong dugon agGGACTACTCCCAATCTGACAAGAAATGTCATCATCAATTTGTACAGAGCTAG
Hydrodamalis gigas agGGACTACTCCCAATCTGACAAGAAATGTCATCATCAATTTGTACAGAGCTAG
Sus scrofa -----
Sus verrucosus -----
Sus cebifrons -----
Physeter macrocephalus agGTTTACTCCAAATCTGACAAGAATTGTCATCATCGGCTGTACAGAGCTAG
Delphinapterus leucas tcACTTTATATAGTGCCTCAGCAATTTACTACCGATTTGCTTTCTAGTATGTC
Lipotes vexillifer agGTTTACTCCAAATCTGACAAGAATTGTCATCATCAGCTGTACAGAGCTAG
Balaena mysticetus agGTTTACTCCGAATCTGACAAGAATTGTCATCATCAGCTGTACAGAGCTAG
Balaenoptera acutorostrata agGTTTACTCCGAATCTGACAAGAATTGTCATCATCAGCTGTACAGAGCTAG
Balaenoptera physalus agGTTTACTCCGAATCTGACAAGAATTGTCATCATCAGCTGTACAGAGCTAG
Balaenoptera bonaerensis agGTTTACTCCGAATCTGACAAGAATTGTCATCATCAGCTGTATAGAGCTAG
Equus asinus agGGACCACCTCTCAATCTGACAAGAAATGTCATCATCAATTTGTACAGAGCTAG
Equus quagga boehmi agGGACCACCTCTCAATCTGACAAGAAATGTCATCATCAATTTGTACAGAGCTAG
Equus przewalskii agGGACCACCTCTCAATCTGACAAGAAATGTCATCATCAATTTGTACAGAGCTAG
Equus caballus agGGACCACCTCTCAATCTGACAAGAAATGTCATCATCAATTTGTACAGAGCTAG
Manis pentadactyla atGGTCTACTCCCAATCTGATGAGAAATGTCATCATGAATTTGTGCAGAGCTAG

586 590 600 610 620 630
 |...|...|...|...|...|...|...|...|...|...|
Homo sapiens TAACATATGATCTAATGAAGGAGGCCTTTGTGAAAAACAACATATTAGCAGgt
Bradypus variegatus ???
Choloepus hoffmanni ???
Mylodon darwini ???
Cyclopes didactylus ???
Dasypus novemcinctus -----
Procavia capensis -----
Elephas maximus TAACATATGACCTGATGAAGGAGACCTTTGTGAAAAACAACCTACTAGCAGct
Mammuthus primigenius TAACATATGACCTGATGAAGGAGACCTTTGTGAAAAACAACCTACTAGCAGct
Loxodonta africana TAACATATGACCTGATGAAGGAGACCTTTGTGAAAAACAACCTACTAGCAGct
Trichechus manatus TAACACATGACCTAATGAAGGAGACCTTTGTGAAAAACAATCTACTGGCAGgt
Dugong dugon TAACACATGACCTAATGAAGGAGACCTTTGTGAAAAACAATCTACTGGCAGgt
Hydrodamalis gigas TAACACATGACCTAATGAAGGAGACCTTTGTGAAAAACAATCTACTGGCAGat
Sus scrofa -----
Sus verrucosus -----
Sus cebifrons -----
Physeter macrocephalus TAACATATGACCTAATGAAGGAGGCCCTTTGTGAAAAACA----ATTAACAGgt
Delphinapterus leucas ACTATCTCATTGACATGCTCCTGCCCTTTGTGAAAAACA----ATTAACAGgt
Lipotes vexillifer TAACATATGACCTAATGAAGGAGACCTTTGTGAAAAACA----ATTAACAGgt
Balaena mysticetus TAACGTATGACCTAATGAAGGAGACCTTTGTGAAAAACA----ATTAACAGgt
Balaenoptera acutorostrata TAACGTATGACCTAATGAAGGAGGCCCTTTGTGAAAAACA----ATTAACAGgt
Balaenoptera physalus TAACGTATGCTTAATGAAGGAGGCCCTTTGTGAAAAACA----ATTAACAGgt
Balaenoptera bonaerensis TAACGTATGACCTAATGAAGGAGGCCCTTTGTGAAAAACA----ATTAACAGgt
Equus asinus TAACCCATGACCTAATGAAGGAGGCCCTTTGTGAAAAACCAACTATTAGCAGgt
Equus quagga boehmi TAACCCATGACCTAATGAAGGAGGCCCTTTGTGAAAAACCAACTATTAGCAGgt
Equus przewalskii TAACCCATGACCTAATGAAGGAGGCCCTTTGTGAAAAACCAACTATTAGCAGgt
Equus caballus TAACCCATGACCTAATGAAGGAGGCCCTTTGTGAAAAACCAACTATTAGCAGgt
Manis pentadactyla TAA--GTATGACCTAATGAAGGAGGCACCTCTAAACAAGA----CTTACCAGct

Exon 5

636 640 650 660 670 680
 |...|...|...|...|...|...|...|...|...|...|
Homo sapiens agATGACGTCCCCTGCCACTTGGTGTGGCTCTTATCGCTGGATTTTGC---

Bradypus variegatus
Choloepus hoffmanni
Mylodon darwini
Cyclopes didactylus
Dasypus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon
Hydrodamalis gigas
Sus scrofa
Sus verrucosus
Sus cebifrons
Physeter macrocephalus
Delphinapterus leucas
Lipotes vexillifer
Balaena mysticetus
Balaenoptera acutorostrata
Balaenoptera physalus
Balaenoptera bonaerensis
Equus asinus
Equus quagga boehmi
Equus przewalskii
Equus caballus
Manis pentadactyla

???
 ???
 ???
 ???

 agGTCATGTGCCCTGCCACTTAGTGTGTTGCTCTTATTGTTGGATTTTGC---
 agATGACGTGCCCTGCCACTTAGTGTCTTCTCTTATCGCTGGATTTTGC---
 agATGACGTGCCCTGCCACTTAGTGTCTTCTCTTATCGCTGGATTTTGC---
 agATGACGTGCCCTGCCACTTAGTGTCTGCTCTTATCGCTGGATTTTGC---
 agATGATGTGCCTTGCCACTTAGTGTCTGCTCTCATGCTGGATTTTGC---
 agATGATGTGCCCTGCCACTCGGTGTCTGCTCTCATGCTGGATTCTGC---
 agATGATGTGCCCTGCCACTCAGTGTCTGCTCTCATGCTGGATTTTGC---

 agATGATGTGCCCTGTCACCTTGTGTCCACTGTTATCGCTGGATTTTGC---
 agATGATGTGCCCGTCACTTTGTGTCCGCTGTTATGCTGGATTTTGC---
 agACGATGTGCCCGTCACTTTGTGTCCGCTGTTATCGCTGGATTTTGC---
 agATGATGTGCCCTGTCACCTTGTGTCCGCTGTTATCGCTGGATTTTGC---
 agATGATGTGCCCTGTCACCTTGTGTCCGCTGTTATCGCTGGATTTTGC---
 agATGATGTGCCCTGTCACCTTGTGTCCGCTGTTATCGCTGGATTTTGC---
 agATGATGTGCCCTGTCACCTTGTGTCCGCTGTTATCGCTGGATTTTGC---
 agATGATGTGCCCTGTCACCTTGTGTCCGCTGTTATCGCTGGATTTTGC---
 agATGATGTACCTTGCCACTTTGTGTCCGCTGTCATCGCCAGATTTTGT---
 agATGATGTACCTTGCCACTTTGTGTCCGCTGTCATCGCCAGATTTTGT---
 agATGATGTACCTTGCCACTTTGTGTCCGCTGTCATCGCCAGATTTTGT---
 agATGATGTACCTTGCCACTTTGTGTCCGCTGTCATCGCCAGATTTTGT---
 agATGATGTACCTTGCCACTTTGTGTCCGCTGTCATCGCCAGATTTTGT---
 agATGATGTACCTTGCCACTTTGTGTCCGCTGTCATCGCCAGATTTTGT---
 agATGATGTACC-TGCCACTTTGTATCAGCTCTTATCACCTGATTTTGTACA

686 690 700 710 720 730
 |...|...|...|...|...|...|...|...|...|...|...|...|
 -GCAACAGCTATGTCCTCCCGGTGGATGTAGTAAAAACCAGATTTATTA
 ???
 ???
 ???
 ???

Homo sapiens
Bradypus variegatus
Choloepus hoffmanni
Mylodon darwini
Cyclopes didactylus
Dasypus novemcinctus
Procavia capensis
Elephas maximus
Mammuthus primigenius
Loxodonta africana
Trichechus manatus
Dugong dugon
Hydrodamalis gigas
Sus scrofa
Sus verrucosus
Sus cebifrons
Physeter microcephalus
Delphinapterus leucas
Lipotes vexillifer
Balaena mysticetus
Balaenoptera acutorostrata
Balaenoptera physalus
Balaenoptera bonaerensis
Equus asinus
Equus quagga boehmi
Equus przewalskii
Equus caballus
Manis pentadactyla

-ACAATTGTTCTGTCTCTCCACTGGACATGGTGAAAAACCAGATTTGTGA
 -ACAATGGTTCGTCTCTCCAGCAGATGTGGTGAAAAACCAGATTTATTA
 -ACAATGGTTCGTCTCTCCAGCAGATGTGGTGAAAAACCAGATTTATTA
 -ACAATGGTTCGTCTCTCCAGCAGATGTGGTGAAAAACCAGATTTATTA
 -ACAACAGTTCGTCTCTCCAGTGGATGTGGTGAAAAACCAGATTTGTTA
 -ACAACAGTTCGTCTCTCCAGTGGATGTGGTGAAAAACCAGATTTGTTA
 -ACAACAGTTCGTCTCTCCAGTGGATGTGGTGAAAAACCAGATTTGTTA

 -ACAACGGTTCGTCTCTCCAGTGGATGCGGTGAAAAACCAGATTTGTGA
 -ACAACGGTTCGTCTCTCCAGTGGATGTGGTGAAAAACCAGATTTGTGA
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 -ACAACGGTTCGTCTCTCCAGTGGATGTGGTGAAAAACCAGATTTGTGA
 -ACAACGGTTCGTCTCTCCAGTGGATGTGGTGAAAAACCAGTCTGTGA
 -ACAACGGTTCGTCTCTCCAGTGGATGTGGTGAAAAACCAGTCTGTGA
 -ACAACAGTTCGTCTCTCCAGTGGACATGGTGAAAAACCACATTTGTTA
 -GCAACAGTTCGTCTCTCCAGTGGACATGGTGAAAAACCACATTTGTTA
 -GCAACAGTTCGTCTCTCCAGTGGACATGGTGAAAAACCACATTTGTTA
 -GCAACAGTTCGTCTCTCCAGTGGACATGGTGAAAAACCACATTTGTTA
 -GCAACAGTTCGTCTCTCCAGTGGACATGGTGAAAAACCACATTTGTTA
 -GCAACAGTTCGTCTCTCCAGTGGACATGGTGAAAAACCACATTTGTTA
 AACAATGTTTTGTCC-CTCTGGTGGATATGGTGAAAAACCAGATTTGTGA

736 740 750 760 770 780

Exon 6

825 830 840 850 860 870
|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
Homo sapiens agGTTGGTACCTTCCTTCTTGCGACTTGGATCCTGGAACGTCATTATGTTTGT
Bradypus variegatus agGTTTGTGCCTTCCTTCTTGCGACTTAGATCCTGGAATGTCATCATGTTTGA
Choloepus hoffmanni agGTTTGTGCCTTCTTCTTGAGACTTGGATCC**TGA**AATGTCATCATGTTTGT
Mylodon darwinii ???
Cyclopes didactylus agATTTGTACCTTCCTTCTTGCAACTAGGATCCTGGAATGTCATCATGTTTGT
Dasypus novemcinctus agTCTTATGCCTTCCTTCTTG**TGA**CTCAGATCCTGGAATGTTGTATGTTTGT
Procavia capensis agGTTTGTACCTTTCCTTCTTG**TGA**CTAAGACTGGGAAGCATCATTAAGTTCAT
Elephas maximus agGCTTGTGCCTTCCTTCTTGCGACTGGGATCCTGGAACGCCATTATGTTTTT
Mammuthus primigenius agGCTTGTGCCTTCCTTCTTGCGACTGGGATCCTGGAACGCCATTATGTTTTT
Loxodonta africana agGCTTGTGCCTTCCTTCTTGCGACTGGGATCCTGGAACGCCATTATGTTTTT
Trichechus manatus agGTTTGTGCCTTCCTTCTTGCGACTCGGTTCTGGAATGTCATTATGTT---T
Dugong dugon agGTTTGTGCCTTCCTTCTTG**TGA**CTCGGATCCTGGAATGTCATTATGTT---T
Hydrodamalis gigas agGTTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGAATGTCATTATGTT---T
Sus scrofa agATTTGTACCTTCCTTCTGG**TGA**CTGGGATCCTGGAACGTCATCCCATTGT
Sus verrucosus agATTTGTACCTTCCTTCTGG**TGA**CTGGGATCCTGGAACGTCATCCCATTGT
Sus cebifrons agATTTGTACCTTCCTTCTGG**TGA**CTGGGATCCTGGAACGTCATCCCATTGT
Physeter microcephalus agATTTGTACCTTCCTTCT**FAGTGA**CTGGGATCCTGGAACATCATCGTGT---GT
Delphinapterus leucas agATTTGTACCTTCCTTCTTG**TGA**CTGGGGTCTGGAACATCATCGTGT---GT
Lipotes vexillifer agATTTGTACCTTCCTTCTTG**TGA**CTGGGATCCTGGAACATCATCGTGT---GT
Balaena mysticetus agATTTGTACCTTCCTTCTTG**TGA**CTGGGATCCTGGAACATCATCGTGT---GT
Balaenoptera acutorostrata agATTTGTACCTTCCTTCTTG**TGA**CTGGGATCCTGGAACATCATCGTGT---GT
Balaenoptera physalus agATTTGTACCTTCCTTCTTG**TGA**CTGGGATCCTGGAACATCATCGTGT---GT
Balaenoptera bonaerensis ???
Equus asinus agATTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGCATGTCATCATGTTTGT
Equus quagga boehmi agATTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGCATGTCATCATGTTTGT
Equus przewalskii agATTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGCATGTCATCATGTTTGT
Equus caballus agATTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGCATGTCATCATGTTTGT
Manis pentadactyla agATTTG----TCCCTTCTTGACCTTGGGTCTCCAGACGTCATTATATTTGT

876 880 890 900 910 920
|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
Homo sapiens GTGCTTTGAACAACCTGAAACGAGAAGTGTCAAAGTCAAGGCAGACTATGGA
Bradypus variegatus GTA---CTGAACAGCTAAAAACAGGAAAGGATGACATCAAGGCGGACTATGGA
Choloepus hoffmanni GTG---TTGAACAGCTAAAAACAGGAAATGATGACATCAAGGCA**ACT**ATGAA
Mylodon darwinii ???
Cyclopes didactylus GTG---TTGAACAGCTAAAAACAGAACTGATGACATCAAGGCAGACTATGGA
Dasypus novemcinctus GTACTTTGAACAGCTGAAAACAAGAATTGATGAAGCCAAGGCAGACTATGGA
Procavia capensis GTGCTTTGAACAGCTGAAAAAAGAATTGATGAAGTCGAGGCAGATGATGGA
Elephas maximus GTGCTCTGAACAGCTGAAAACAAGAATTGATGAAGTCAAGGCAGACTATGGG
Mammuthus primigenius GTGCTCTGAACAGCTGAAAACAAGAATTGATGAAGTCAAGGCAGACTATGGG
Loxodonta africana GTGCTCTGAACAGCTGAAAACAAGAATTGATGAAGTCAAGGCAGACTATGGG
Trichechus manatus GTGCTTTGAA**TAC**CTGAAAACGAGAATTGATGAAGTCGAGGGAGACTATGGA
Dugong dugon GTGCTTTGAACAGCTGAAAACGAGAATTGATGAAGTCGAGGCAGACTATGGA
Hydrodamalis gigas GTGCTTTGAACAGCTGAAAACGAGAATTGATGAAGTCGAGGCAGACTATGGA
Sus scrofa GTGCTTTGAACAGCTGAAAACAAGAGTTGATGGAGTCAAGGCAGACTGTGGA
Sus verrucosus GTGCTTTGAACAGCTGAAAACAAGAGTTGATGGAGTCAAGGCAGACTGTGGA
Sus cebifrons GTGCTTTGAACAGCTGAAAACAAGAGTTGATGGAGTCAAGGCAGACTGTGGA
Physeter microcephalus GTGCCTTGAACAGCTGAAGCGAGAATTGCTGAAGTCGAGGCAGACCATGGA
Delphinapterus leucas GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTTGAGGCAGACCATGGA
Lipotes vexillifer GTGCTTTGAACAGCTGAAG**TGA**GAATTGATGAAGTTGAGGCAGACCATGGA
Balaena mysticetus GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCGAGGCAGACCATGGA
Balaenoptera acutorostrata GTGCTCTGAACAGCTGACGCGAGAATTGATGAAGTCGAGGCAGACCATGGA
Balaenoptera physalus GTGCTTTGAACAGCTGAAGMGAGAATTGATGAAGTCGAGGCAGACCATGGA
Balaenoptera bonaerensis ???
Equus asinus GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCAAGGCAGACCATGGA

<i>Equus quagga boehmi</i>	GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCAACGCAGACCATGGA
<i>Equus przewalskii</i>	GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCAACGCAGACCATGGA
<i>Equus caballus</i>	GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCAACGTGACAGACCAT
<i>Manis pentadactyla</i>	GTGCTCTGAACAAGGAAAA TGA AAATTGACGAACTCAAGG -----
	925 930 940 950

<i>Homo sapiens</i>	CTGTGCCACA-----TAA
<i>Bradypus variegatus</i>	CTGTGCCACAGCTTCAGGAAAAGAATG ----- TAA
<i>Cyclopes didactylus</i>	CTTTGCCACA ACTTCAGGAAAAGGATG ----- TAA
<i>Choloepus hoffmanni</i>	CTGTGTCACAGCTTCAGGAAAAGAATGTTAA
<i>Mylodon darwini</i>	????????????????????????????????
<i>Dasypus novemcinctus</i>	CTGTGCTACA-----TAA
<i>Procavia capensis</i>	CTGTGCCACA-----TAA
<i>Elephas maximus</i>	CTGTACCACA-----TAA
<i>Mammuthus primigenius</i>	CTGTACCACA-----TAA
<i>Loxodonta africana</i>	CTGTACCACA-----TAA
<i>Trichechus manatus</i>	CTGTGCCACA-----TAA
<i>Dugong dugon</i>	CTGTGCCACA-----TAA
<i>Hydrodamalis gigas</i>	CTGTGCCACA-----TAA
<i>Sus scrofa</i>	CTGTGCTGCA-----TAA
<i>Sus verrucosus</i>	CTGTGCTGCA-----TAA
<i>Sus cebifrons</i>	CTGTGCTGCA-----TAA
<i>Physeter microcephalus</i>	CTGTGCTACC-----TAA
<i>Delphinapterus leucas</i>	CTGTGCTACC-----TAA
<i>Lipotes vexillifer</i>	CTGTGCTACC-----TAA
<i>Balaena mysticetus</i>	CTGTGCTACC-----TAA
<i>Balaenoptera acutorostrata</i>	CTGTGCTACC-----TAA
<i>Balaenoptera physalus</i>	CTGTGCTACC-----TAA
<i>Balaenoptera bonaerensis</i>	????????????????????????????????
<i>Equus asinus</i>	CTGTGCCACA-----TAA
<i>Equus quagga boehmi</i>	CTGTGCCACA-----TAA
<i>Equus przewalskii</i>	CTGTGCCACA-----TAA
<i>Equus caballus</i>	CTGTGCCACA-----TAA
<i>Manis pentadactyla</i>	CTGTGTCATA-----TAA

Figure S1. Exon alignments depicting deleterious mutations found in *UCPI* sequences of placental mammal taxa. The intact human *UCPI* sequence is provided for reference. Mutated initiation codons (green), premature stop codons (red), frameshift insertions (turquoise), deletions (dashes), frameshift deletions (pink), mutated splice sites (yellow), and mutated stop codons (teal) are indicated. Note insert from ~800 bp upstream of *UCPI* in exon 4 of beluga (grey). Missing data are denoted by question marks.

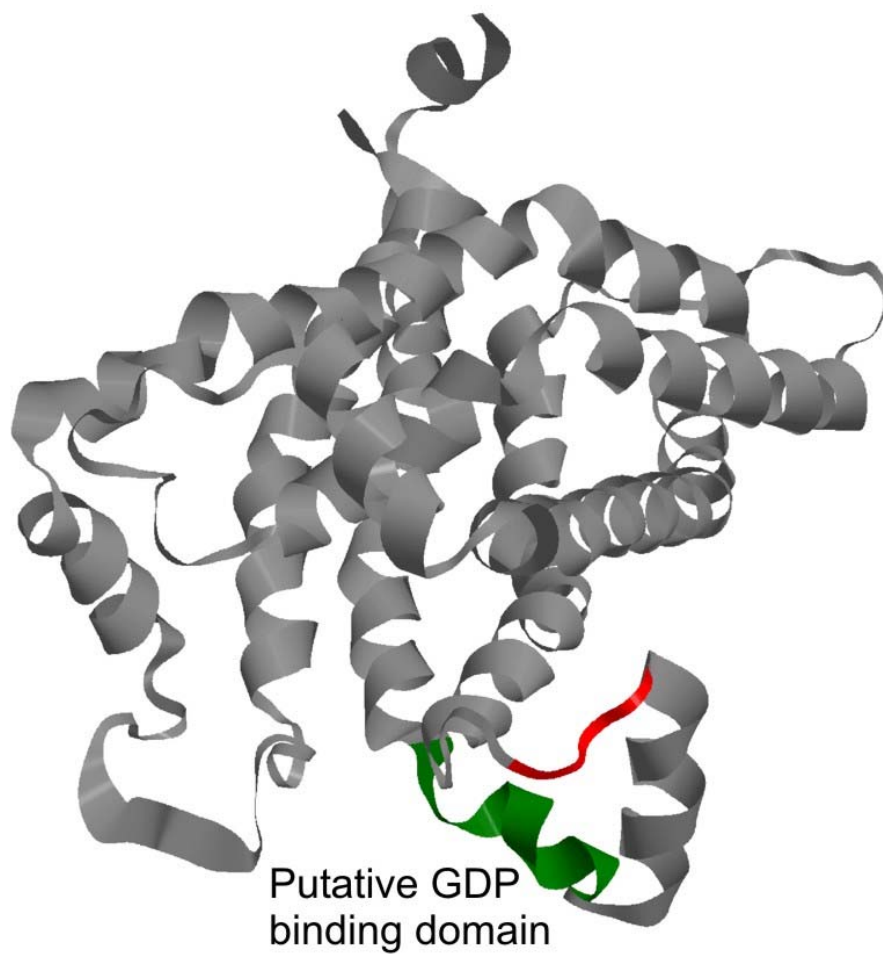


Figure S2. Ribbon diagram of residues 13 to 304 of human UCP1 (UniProt accession number P25874) structurally modelled by SWISSMODEL. Residues highlighted in red denote the position of a four amino acid deletion in the third matrix loop of the camel (*Camelus ferus*) UCP1 protein which borders the putative GDP-binding domain (green).

<i>Pantholops hodgsonii</i>	M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y
<i>Ovis aries</i>	M	E	R	S	A	R	E	I	C	L	N	F	T	V	V	L	I	T	V	M	L	I	W	L	L	V	R	S	Y	Q	Y
<i>Capra hircus</i>	M	E	R	S	T	R	E	I	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y
<i>Capra aegagrus</i>	M	E	R	S	T	R	E	I	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y
<i>Bubalus bubalis</i>	M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y
<i>Bison bison</i>	M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y
<i>Bos grunniens</i>	M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y
<i>Bos indicus</i>	M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y
<i>Bos taurus</i>	M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y

Figure S3. Amino acid alignment of vertebrate sarcolipin. The mutated start codons (ATG→CTG) in the two-toed sloth (*Choleopus hoffmannii*) and brown-throated sloth (*Bradypus variegatus*) were verified from the NCBI sequence read archive and polymerase chain reaction amplification, respectively. Amino acid deletions in the Equid lineage are highlighted in red. Note that two sarcolipin gene copies were found in the *Xenopus tropicalis*, *Monodelphis domestica*, and *Physeter macrocephalus* assemblies. Missing data denoted by question marks.

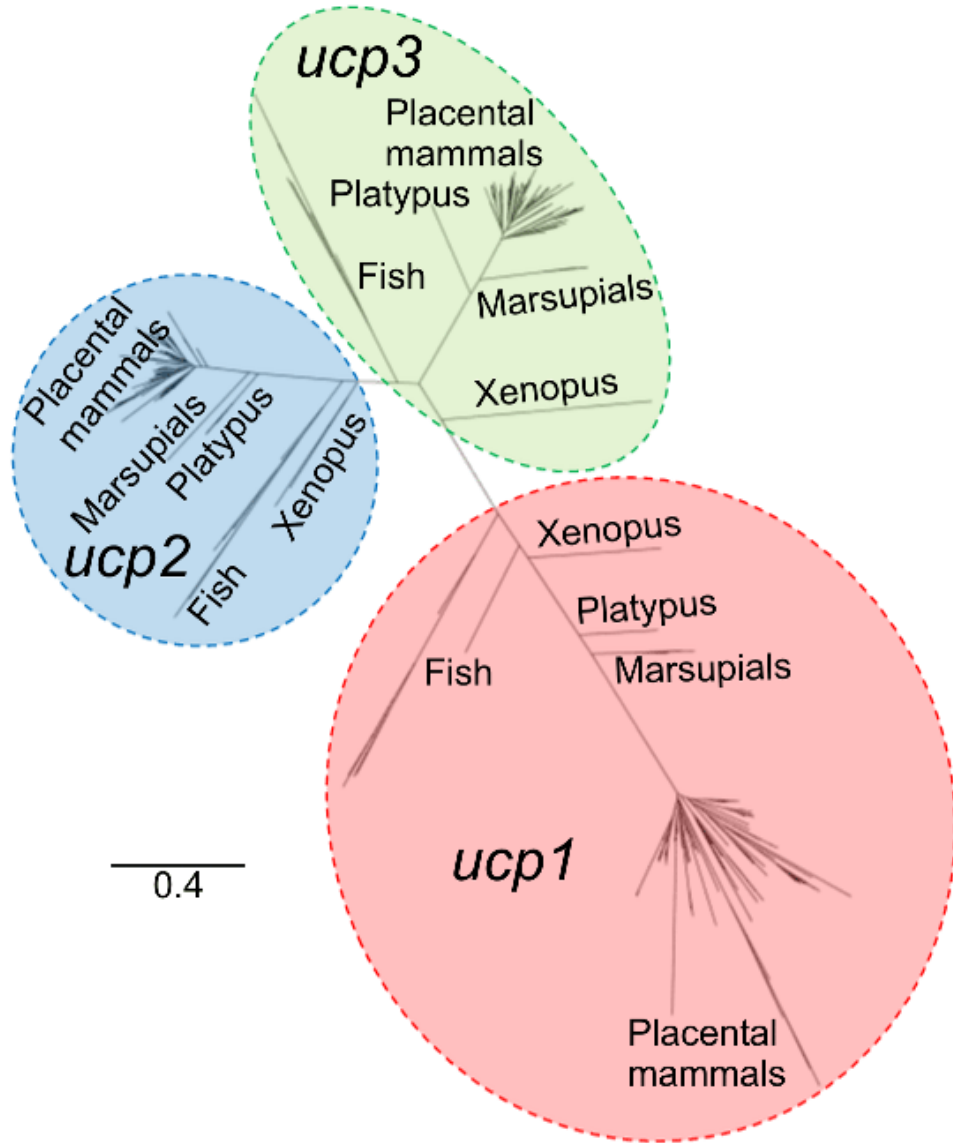


Figure S4. Schematic maximum-likelihood tree of *ucp* sequences used in this study (N=400). Branch lengths denote number of substitutions per site.

Table S1. GenBank accession numbers of species used in this study.

<i>Species name</i>	<i>ucp1 Source</i>	<i>ucp2 Source</i>	<i>ucp3 Source</i>	<i>sln Source</i>
Osteichthyes				
<i>Astyanax mexicanus</i>	APWO01100404.1 APWO01100403.1	APWO01075838.1	APWO01075838.1	Missing
<i>Cyprinus carpio</i>	AY461434.2	AJ243486.1	AY505343.1	Missing
<i>Danio rerio</i>	CABZ01073633.1 CABZ01073634.1	CABZ01017713.1	CABZ01012840.1 CABZ01017713.1	NM_001302745.1
<i>Latimeria chalumnae</i>	AFYH01226130.1 AFYH01226129.1	BAHO01089108.1 AFYH01155949.1	Missing	AFYH01102483.1
<i>Lepisosteus oculatus</i>	AHAT01015452.1	AHAT01003069.1	AHAT01003069.1	AHAT01003063.1
<i>Takifugu rubripes</i>	CAAB02001533.1	CAAB02000575.1	CAAB02000575.1	CAAB02007733.1
<i>Xiphophorus maculatus</i>	AGAJ01004818.1 AGAJ01004819.1	AGAJ01021216.1	AGAJ01021216.1	AGAJ01040366.1

Amphibia

<i>Xenopus tropicalis</i>	AAMC02004107.1	NM_203848.1	NM_001095178.1	AAMC02008311.1 AAMC02008313.1
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Montremata

<i>Ornithorhynchus anatinus</i>	NW_001794248.1	AAPN01056149.1 AAPN01056148.1 AAPN01056147.1 AAPN01382618.1	AAPN01178604.1 AAPN01178605.1	AAPN01035927.1
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Marsupialia

<i>Monodelphis domestica</i>	AAFR03015618.1	AAFR03050488.1	AAFR03050488.1	AAFR03009954.1 AAFR03043596.1
<i>Macropus eugenii</i>	ABQO020217652.1 ABQO020162103.1 ABQO020106554.1 ABQO020051005.1 ABQO021106434.1	ABQO020850797.1 ABQO020012246.1	ABQO020339531.1 ABQO020395080.1 ABQO020450629.1 ABQO020707648.1 ABQO020652099.1	ABQO020365261.1
<i>Sarcophilus harrisii</i>	AEFK01228715.1 AEFK01215941.1	AFEY01422591.1	AFEY01409403.1 AFEY01344314.1 AFEY01422590.1	AFEY01139015.1
<i>Sminthopsis crassicaudata</i>	EF622232.1	Missing	Missing	Missing

Xenarthra

<i>Choloepus hoffmanni</i>	ABVD02364175.1	ABVD01312484.1	ABVD02315747.1	ABVD02284044.1
<i>Bradypus variegatus</i>	This study	Missing	Missing	This study
<i>Cyclopes didactylus</i>	This study	Missing	Missing	Missing
<i>Dasybus novemcinctus</i>	AAGV03181320.1	AAGV03150769.1	AAGV03292997.1 AAGV03292996.1	AAGV03173212.1
<i>Myiodon darwini</i>	SRX327588	SRX327588 SRX327589	SRX327588 SRX327589 SRX465052	SRX327588

Afrotheria

<i>Chrysochloris asiatica</i>	AMDV01244955.1 AMDV01244956.1	AMDV01302651.1	AMDV01302653.1	AMDV01027313.1
<i>Dugong dugon</i>	This study	This study	This study	This study
<i>Echinops telfairi</i>	AAIY02209271.1	AAIY02169770.1	AAIY02169772.1	AAIY02172361.1
<i>Elephantulus edwardii</i>	AMGZ01097263.1	AMGZ01085574.1	AMGZ01085576.1; AMGZ01085575.1	AMGZ01226582.1
<i>Elephas maximus</i>	This study	ERX334764 ERX334765	ERX334764 ERX334765	ERX334764 ERX334765
<i>Hydrodamalis gigas</i>	This study	This study	This study	This study
<i>Loxodonta africana</i>	AAGU03034821.1	AAGU03079300.1	AAGU03079300.1	AAGU03058675.1
<i>Mammuthus primigenius</i>	ERP008929	ERP008929	ERP008929	ERP008929
<i>Orycteropus afer</i>	ALYB01104541.1 ALYB01104543.1	ALYB01103367.1	ALYB01103365.1	ALYB01161748.1
<i>Procapra capensis</i>	ABRQ02143236.1	ABRQ02011699.1	ABRQ02011697.1	ABRQ02037372.1
<i>Trichechus manatus latirostris</i>	AHIN01109623.1 AHIN01109624.1	AHIN01084584.1	AHIN01084587.1	AHIN01088368.1

Laurasiatheria

<i>Acinonyx jubatus</i>	LLWD01000416.1	LLWD01000509.1	LLWD01000509.1	LLWD01000070.1
<i>Ailuropoda melanoleuca</i>	ACTA01016457.1	ACTA01146014.1	ACTA01130015.1	ACTA01090054.1
<i>Balaena mysticetus</i>	This study	Missing	Missing	Missing
<i>Balaenoptera acutorostrata</i>	ATDI01065547.1	ATDI01094623.1	ATDI01094627.1	ATDI01043778.1
<i>Balaenoptera bonaerensis</i>	BAUQ01197845.1 BAUQ01696173.1 BAUQ01341929.1	BAUQ01410165.1	BAUQ01284797.1	BAUQ01341977.1
<i>Balaenoptera physalus</i>	SRX323050	SRX323050	SRX323050	SRX323050
<i>Bison bison</i>	JPYT01100523.1	JPYT01642782.1	JPYT01019071.1	JPYT01254401.1
<i>Bos grunniens</i>	AGSK01075302.1	AGSK01058532.1 [Misassembled]	AGSK01132661.1 AGSK01132663.1 [Misassembled]	AGSK01136949.1
<i>Bos indicus</i>	AGFL01142555.1 AGFL01142556.1	AGFL01131821.1	AGFL01131836.1 AGFL01131835.1	AGFL01128732.1
<i>Bos taurus</i>	DAAA02044420.1 DAAA02044421.1	AAFC03092999.1	NM_174210.1	AAFC03004956.1
<i>Bubalus bubalis</i>	AWWX01630119.1	AWWX01596091.1	AWWX01532946.1	AWWX01539210.1
<i>Camelus ferus</i>	AGVR01051296.1	AGVR01023127.1	AGVR01023127.1	AGVR01041834.1
<i>Canis lupus familiaris</i>	NM_001003046.1	AAEX03012810.1	NM_001003047.1	AOCS01147984.1
<i>Capra aegagrus</i>	CBYH010071014	CBYH010041467.1	CBYH010041467.1	JXYW01145092.1
<i>Capra hircus</i>	AJPT01162993.1	JN986832.1	AJPT01151377.1	AJPT01148506.1
<i>Capreolus capreolus</i>	CCMK010104759.1 CCMK012865005.1 CCMK010278719.1	CCMK010138775.1	CCMK010008945.1	CCMK010366203.1
<i>Ceratotherium simum</i>	AKZM01017598.1	AKZM01032604.1	AKZM01032604.1	AKZM01019766.1
<i>Choeropsis liberiensis</i>	This study	This study	This study	Missing
<i>Coelodonta antiquitatis</i>	This study	This study	This study	This study
<i>Condylura cristata</i>	AJFV01047153.1	AJFV01042335.1	AJFV01042336.1	AJFV01057960.1
<i>Delphinapterus leucas</i>	This study	This study	This study	Missing
<i>Diceros bicornis</i>	This study	Missing	Missing	Missing

	This study	This study	This study	This study
<i>Dicerorhinus sumatrensis</i>	This study	This study	This study	This study
<i>Eidolon helvum</i>	AWHC01029981.1	AWHC01218500.1	AWHC01218502.1 AWHC01218501.1	AWHC01196379.1
<i>Eptesicus fuscus</i>	ALEH01005956.1	ALEH01084492.1	ALEH01084495.1; ALEH01084494.1	ALEH01120006.1
<i>Equus asinus</i>	SRX290673 SRX290675 SRX290677	SRX290673 SRX290675 SRX290677	SRX290673 SRX290675 SRX290677	ERX607030 ERX607036 ERX607001
<i>Equus caballus</i>	AAWR02018851.1	AAWR02018167.1	AAWR02018167.1	AAWR02020714.1
<i>Equus przewalskii</i>	ATBW01036321.1 ATBW01036320.1	ATBW01058700.1 ATBW01058701.1	ATBW01058699.1	ATBW01057363.1
<i>Equus quagga boehmi</i>	This study	Missing	Missing	Missing
<i>Erinaceus europaeus</i>	AMDU01193160.1 AMDU01193161.1 AMDU01193162.1	AANN01307683.1	AMDU01063251.1 AMDU01063252.1 AMDU01063253.1	AANN01527796.1
<i>Felis catus</i>	ACBE01132808.1 ACBE01132809.1	AANG02100994.1	AANG02100994.1	ACBE01332355.1
<i>Leptonychotes weddellii</i>	APMU01141180.1 APMU01115166.1	APMU01037391.1	APMU01037393.1	APMU01115847.1
<i>Lipotes vexillifer</i>	AUPI01000024.1 AUPI01000025.1	AUPI01017650.1	AUPI01017651.1	AUPI01060449.1
<i>Manis pentadactyla</i>	JPTV01131901.1	JPTV01099221.1	JPTV01146288.1	JPTV01082441.1
<i>Megaderma lyra</i>	AWHB01348443.1 AWHB01348444.1	AWHB01174334.1 AWHB01174333.1	AWHB01278871.1 AWHB01278872.1	AWHB01215178.1
<i>Mustela putorius furo</i>	AGTQ01041845.1	AGTQ01009941.1	AGTQ01009939.1	AEYP01061363.1
<i>Myotis brandtii</i>	ANKR01273868.1 ANKR01273869.1	ANKR01210024.1	ANKR01210023.1	ANKR01318413.1
<i>Myotis davidii</i>	ALWT01125743.1	ALWT01314732.1 ALWT01314733.1	ALWT01314729.1	ALWT01118440.1
<i>Myotis lucifugus</i>	AAPE02001462.1	AAPE02040816.1	AAPE02040815.1 AAPE02040816.1	AAPE02047638.1
<i>Neovison vison</i>	EU360729.1	EU360730.1	Missing	Missing
<i>Odobenus rosmarus</i>	ANOP01028105.1	ANOP01000820.1	ANOP01000821.1	ANOP01017035.1
<i>Orcinus orca</i>	ANOL02004931.1 (Deleted gene - Synteny analysis)	ANOL02054064.1	ANOL02054065.1	ANOL02064465.1
<i>Ovis aries</i>	JN604985.1	CBYI010055183.1	CBYI010055184.1	ACIV011354444.1
<i>Panthera tigris altaica</i>	ATCQ01112915.1	ATCQ01048793.1	ATCQ01048791.1	ATCQ01029167.1
<i>Panthera uncia</i>	SRX273036	SRX273036	SRX273036	SRX273036
<i>Pantholops hodgsonii</i>	AGTT01188813.1	AGTT01169141.1	AGTT01169136.1	AGTT01037849.1
<i>Phoca vitulina</i>	AB921529.1	Missing	Missing	Missing
<i>Physeter macrocephalus</i>	AWZP01062081.1	AWZP01062236.1	AWZP01062236.1	AWZP01043685.1 AWZP01078901.1
<i>Pteropus alecto</i>	ALWS01011689.1	ALWS01169197.1	ALWS01169198.1 ALWS01169197.1	ALWS01050977.1
<i>Pteropus vampyrus</i>	ABRP02126915.1	ABRP02138358.1	ABRP02138358.1	ABRP02100214.1
<i>Rhinoceros unicornis</i>	This study	Missing	Missing	Missing
<i>Rhinolophus ferrumequinum</i>	AWHA01040305.1 AWHA01040304.1	AWHA01096410.1	AWHA01092973.1 AWHA01092975.1	AWHA01130749.1

<i>Sorex araneus</i>	AALT02056093.1	AALT02155451.1	AALT02155448.1 AALT02155449.1	AALT02042376.1
<i>Suncus murinus</i>	AB244816.1	Missing	Missing	Missing
<i>Sus cebriifrons</i>	ERX149172	ERX149172	ERX149172	ERX149172
<i>Sus scrofa</i>	DQ372918.1	NM_214289.1	NM_214049.1	AJKK01191606.1
<i>Sus verrucosus</i>	ERX1054048- ERX1054067	ERX1054048- ERX1054067	ERX1054048- ERX1054067	ERX1054048- ERX1054067
<i>Tapirus indicus</i>	This study	This study	This study	This study
<i>Tursiops truncatus</i>	ABRN02199412.1 (Deleted gene - Synteny analysis)	ABRN02011700.1	ABRN02011697.1	ABRN02379235.1
<i>Ursus maritimus</i>	AVOR01014285.1	AVOR01090225.1	AVOR01090228.1	AVOR01032007.1
<i>Vicugna pacos</i>	ABRR02134987.1	ABRR02047266.1	ABRR02047266.1	ABRR02146164.1

Euarchontoglires

<i>Aotus nancymaae</i>	JYKP01215429.1	JYKP01068224.1	JYKP01068227.1	JYKP01155709.1
<i>Apodemus sylvaticus</i>	LIPJ01014497.1 LIPJ01184746.1	LIPJ01041350.1	LIPJ01000433.1	LIPJ01004285.1
<i>Callithrix jacchus</i>	ACFV01002817.1 ACFV01002818.1	ACFV01035072.1	ACFV01035065.1 ACFV01035066.1 ACFV01035067.1	JRUL010233331.1
<i>Cavia aperea</i>	AVPZ01000778.1	AVPZ01000005.1	AVPZ01000005.1	AVPZ01000041.1
<i>Cavia porcellus</i>	AAKN02011801.1	AAKN02051191.1	AAKN02051190.1	AAKN02053822.1
<i>Cercocebus atys</i>	JZLG01060688.1	JZLG01056565.1	JZLG01056561.1	JZLG01010897.1
<i>Chinchilla lanigera</i>	AGCD01027651.1 AGCD01027652.1	AGCD01015245.1	AGCD01015243.1	AGCD01012756.1
<i>Chlorocebus sabaues</i>	AQIB01017419.1	AQIB01134135.1	AQIB01134133.1	AQIB01093034.1
<i>Colobus angolensis</i>	JYKR01122839.1 JYKR01122838.1 JYKR01122837.1	JYKR01062401.1	JYKR01062397.1	JYKR01171003.1
<i>Cricetulus griseus</i>	AFTD01128394.1	AFTD01106142.1	AFTD01106142.1	APMK01041211.1
<i>Daubentonia madagascariensis</i>	AGTM011584996.1 AGTM011708528.1 AGTM012010142.1 AGTM011594144.1	AGTM010366851.1 AGTM012942927.1 AGTM010409851.1 AGTM011996042.1 AGTM011949823.1	AGTM010372645.1 AGTM011680642.1	AGTM011625695.1
<i>Dicrostonyx groenlandicus</i>	AF515781.1	Missing	Missing	Missing
<i>Dipodomys ordii</i>	ABRO02057411.1	ABRO02020382.1	ABRO02020382.1	ABRO02010842.1
<i>Eulemur flavifrons</i>	LGHW01000184.1	LGHW01005764.1	LGHW01005764.1	LGHW01001219.1
<i>Eulemur macaco</i>	LGHX01000184.1	LGHX01005675.1	LGHX01005675.1	LGHX01001209.1
<i>Fukomys damarensis</i>	AYUG01151055 AYUG01151056	AYUG01134562.1 AYUG01134563.1	AYUG01134566.1 AYUG01134565.1	AYUG01150094.1
<i>Galeopterus variegatus</i>	JMZW01045216.1 JMZW01045217.1	JMZW01159785.1 JMZW01159786.1	JMZW01159781.1	JMZW01093471.1
<i>Gorilla gorilla gorilla</i>	NW_004002547.1	NW_004005093.1	NW_004005093.1	CABD030081410.1
<i>Heterocephalus glaber</i>	AFSB01162372.1	AFSB01032172.1	AFSB01032171.1	AFSB01080593.1
<i>Homo sapiens</i>	NG_012139.1	U82819.1	U84763.1	U96094.1

<i>Jaculus jaculus</i>	AKZC01091543.1	AKZC01100636.1 AKZC01100637.1	AKZC01100628.1 AKZC01100629.1 AKZC01100630.1 AKZC01100632.1	AKZC01057088.1
<i>Macaca fascicularis</i>	CAEC01514737.1	AEHL01150243.1	AEHL01150254.1; AEHL01150253.1	CAEC01142624.1
<i>Macaca mulatta</i>	AANU01271750.1	AANU01189618.1	AANU01189616.1	AEHK01077455.1
<i>Macaca nemestrina</i>	JZLF01028562.1	JZLF01057018.1	JZLF01057022.1	JZLF01029339.1
<i>Mandrillus leucophaeus</i>	JYKQ01107155.1 JYKQ01107156.1	JYKQ01166891.1	JYKQ01166885.1	JYKQ01102438.1
<i>Marmota marmota</i>	CZRN01000015.1	CZRN01000004.1	CZRN01000004.1	CZRN01000026.1
<i>Mesocricetus auratus</i>	NM_001281332.1	APMT01084061.1	APMT01084061.1	APMT01047702.1
<i>Microcebus murinus</i>	ABDC01082367.1	ABDC01179060.1	ABDC01179052.1 ABDC01179053.1	ABDC02015405.1
<i>Microtus agrestis</i>	LIQJ01004042.1	LIQJ01014426.1	LIQJ01014426.1	LIQJ01000448.1
<i>Microtus ochrogaster</i>	AHZW01157106.1	AHZW01107615.1	AHZW01107615.1	AHZW01020748.1
<i>Mus musculus</i>	CAAA01024310.1	CAAA01006333.1	AAHY01064811.1	AAHY01078220.1
<i>Myodes glareolus</i>	LIPI01003929.1	LIPI01011290.1	LIPI01011290.1	LIPI01022398.1
<i>Nannospalax galili</i>	AXCS01128925.1	AXCS01188952.1	AXCS01188951.1	AXCS01045434.1
<i>Nasalis larvatus</i>	JMHX01319533.1	JMHX01319543.1	CM003003.1	JMHX01319543.1
<i>Nomascus leucogenys</i>	ADFV01177960.1 ADFV01177959.1	ADFV01111583.1	ADFV01111578.1 ADFV01111580.1	ADFV01056290.1
<i>Ochotona dauurica</i>	AB283043.1	Missing	Missing	Missing
<i>Ochotona princeps</i>	ALIT01060999.1	AAYZ01137505.1	AAYZ01237828.1	AAYZ01218708.1
<i>Octodon degus</i>	AJSA01193670.1 AJSA01193671.1	AJSA01174660.1	AJSA01174659.1 AJSA01174660.1	AJSA01031476.1
<i>Oryctolagus cuniculus</i>	NM_001171077.1	AAGW02008192.1	AAGW02008192.1	AAGW02009195.1
<i>Otolemur garnettii</i>	AAQR03074138.1	AAQR03001165.1	AAQR03001162.1	AAQR03181843.1
<i>Pan paniscus</i>	AJFE01070904.1		AJFE01047917.1	AJFE01100549.1
<i>Pan troglodytes</i>	AACZ03032212.1 AACZ03032211.1	AACZ03079660.1	AADA01222375.1	AADA01279835.1
<i>Papio anubis</i>	AHZZ01043343.1	AHZZ01024419.1	AHZZ01024424.1	AHZZ01040054.1
<i>Papio hamadryas</i>	PreEnsembl	Missing	Missing	Missing
<i>Peromyscus maniculatus</i>	AYHN01134223.1 AYHN01134224.1	AYHN01057054.1	AYHN01057054.1	AYHN01010930.1
<i>Phodopus sungorus</i>	AF271263.1	AF271264.1	AF271265.2	Missing
<i>Pongo abelii</i>	ABGA01062109.1 ABGA01062111.1	ABGA01223176.1 ABGA01223175.1	ABGA01223178.1	ABGA01383648.1
<i>Propithecus coquereli</i>	JZKE01017273.1 JZKE01017272.1 JZKE01017271.1	JZKE01114303.1	JZKE01114303.1	JZKE01236593.1
<i>Rattus norvegicus</i>	AAHX01097782.1	AAHX01007471.1	AABR06007535.1	AAHX01054738.1
<i>Rhinopithecus roxellana</i>	JABR01098768.1	JABR01036514.1	JABR01036511.1	JABR01016757.1
<i>Saimiri boliviensis</i>	AGCE01051213.1	AGCE01022106.1	AGCE01022109.1	AGCE01023439.1
<i>Spermophilus tridecemlineatus</i>	AGTP01049379.1	AAQQ01149310.1	AGTP01028875.1	AAQQ01672914.1
<i>Tarsius syrichta</i>	ABRT02355485.1 ABRT02355484.1	ABRT02455082.1	ABRT02455086.1 ABRT02455085.1	ABRT02333009.1
<i>Tupaia belangeri chinensis</i>	ALAR01031044.1	ALAR01010153.1	ALAR01010154.1	ALAR01067265.1

Table S2. Likelihood ratio tests for *ucp1* CODEML models. The M2 pseudogene branch model was tested against individual branch models (M2), with additional branch categories for the camel, pygmy hippopotamus, stem rhinoceros, and stem pinniped branches. The pseudogene branch model (M2) analyses with and without the stem placental branch as its own category were tested against each other showing that ω of the stem placental branch ($\omega = 0.6333$) is significantly different than all placental branches with functional *ucp1* ($\omega = 0.1591$). Branch categories for the pseudogene analysis included non-mammalian vertebrates, non-placental mammals, placental branches with intact *ucp1* loci, individual transitional branches (M2 ω values displayed in Fig. 3), and pseudogenic branches. Site and branch-site models were performed with pseudogenes pruned from the dataset. The site model M2a revealed no sites under positive selection and no difference from the null model M1a. The branch site model A was performed to identify sites on the stem placental branch that may have contributed to the gain of NST function. While eight sites were found to have a posterior probability >0.95 , model A was not significantly different from the corresponding null model where ω is fixed at 1 for foreground branch sites in the positive selection site category. Site and branch-site model parameters include the proportion of sites (p_0, p_1, p_2, p_{2a} , and p_{2a}) and omega values ($\omega_0, \omega_1, \omega_2, \omega_{2a}$ and ω_{2b}) for all site classes.

LRT models	Parameter estimates	2 Δ l	df	p-value	Sites (posterior probability >0.95)
Branch models					
M2 pseudogene vs. M2 camel branch	<p>M2 pseudogene:</p> <ul style="list-style-type: none"> ω functional non-mammal branches = 0.0501 ω functional non-placental branches = 0.1238 ω functional placental branches = 0.1591 ω pseudogenic placental branches = 0.9443 <p>M2 camel branch:</p> <ul style="list-style-type: none"> ω functional non-mammal branches = 0.0501 ω functional non-placental branches = 0.1238 ω functional placental branches = 0.1585 ω pseudogenic placental branches = 0.9438 ω <i>C. ferus</i> = 1.0330 	4.66614	1	0.030762959	
M2 pseudogene vs. M2 pygmy hippo branch	<p>M2 pseudogene:</p> <ul style="list-style-type: none"> ω functional non-mammal branches = 0.0501 ω functional non-placental branches = 0.1238 ω functional placental branches = 0.1591 ω pseudogenic placental branches = 0.9443 <p>M2 pygmy hippo branch:</p> <ul style="list-style-type: none"> ω functional non-mammal branches = 0.0501 ω functional non-placental branches = 0.1238 ω functional placental branches = 0.1585 ω pseudogenic placental branches = 0.9439 ω <i>C. liberiensis</i> = 0.2530 	1.45469	1	0.227776571	

M2 pseudogene vs. M2 stem pinniped branch	M2 pseudogene: ω functional non-mammal branches = 0.0501 ω functional non-placental branches = 0.1238 ω functional placental branches = 0.1591 ω pseudogenetic placental branches = 0.9443 M2 pinniped branch: ω functional non-mammal branches = 0.0501 ω functional non-placental branches = 0.1238 ω functional placental branches = 0.1580 ω pseudogenetic placental branches = 0.9439 ω stem pinniped branch = 0.2866	0.074304175	1	0.074304175
M2 pseudogene vs. M2 stem rhinoceros branch	M2 pseudogene: ω functional non-mammal branches = 0.0501 ω functional non-placental branches = 0.1238 ω functional placental branches = 0.1591 ω pseudogenetic placental branches = 0.9443 M2 stem rhinoceros branch: ω functional non-mammal branches = 0.0501 ω functional non-placental branches = 0.1238 ω functional placental branches = 0.1585 ω pseudogenetic placental branches = 0.9438 ω stem rhinoceros branch = 0.2851	1.459344	1	0.227034526
M2 pseudogene vs. M2 stem placental branch	M2 pseudogene: ω functional non-mammal branches = 0.0501 ω functional non-placental branches = 0.1238 ω functional placental branches = 0.1591 ω pseudogenetic placental branches = 0.9443 M2 stem placental branch: ω functional non-mammal branches = 0.0502 ω functional non-placental branches = 0.1183 ω functional placental branches = 0.1536 ω pseudogenetic placental branches = 0.9437 ω stem placental branch = 0.6333	36.676754	1	1.39432E-9

Site models

M1a vs. M2a	M1a: $p_0 = 0.92037$ $p_1 = 0.07963$ $\omega_0 = 0.11041$ $\omega_1 = 1.00000$ M2a: $p_0 = 0.92037$ $p_1 = 0.05616$ $p_2 = 0.02347$ $\omega_0 = 0.11038$ $\omega_1 = 1.00000$ $\omega_2 = 1.00004$	0	2	1	None
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Branch-site models

Model A null vs. Model A - stem placental branch	Model A null: $p_0 = 0.47004$ $p_1 = 0.04251$ $p_{2a} = 0.44702$ $p_{2b} = 0.04043$ ω_0 & ω_{2a} background = 0.10531 ω_1 & ω_{2b} background = 1.00000 ω_0 foreground = 0.10531 ω_1, ω_{2a} & ω_{2b} foreground = 1.00000 Model A: $p_0 = 0.54398$ $p_1 = 0.04910$ $p_{2a} = 0.37323$ $p_{2b} = 0.03369$ ω_0 & ω_{2a} background = 0.10532	0.63447	1	0.425720414	15L, 22S, 76K, 124L, 153I, 170T, 224I, 261M
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ω_1 & ω_{2b} background = 1.00000
 ω_0 foreground = 0.10532
 ω_1 foreground = 1.00000
 ω_{2a} & ω_{2b} foreground = 1.40522

Table S3. *UCPI* inactivation dates (millions of years ago; Ma) within the placental mammal lineage. Inactivation date ranges were calculated using the two sets of formulas specified by Meredith *et al.* (26) with branch model CODEML analyses using species divergences and branch lengths arising from our 51 kb maximum likelihood species timetree analysis (data file S2). The first dating method assumes the rate of “synonymous substitution are neutral and equal on functional and pseudogene branches” while the second assumes “the rate synonymous substitution on functional branches is non-neutral and 70% of the rate of synonymous substitution on pseudogene branches”. Inactivation date calculations utilized ω values for placental branches with functional *ucp1* ($\omega=0.1591$), transitional branches (ω values displayed in Fig. 3), and pseudogenic branches ($\omega=0.9435$). As ω values calculated for *Dasyopus novemcinctus*, *Manis pentadactyla*, and the stem cetacean branch ($\omega=1.0567$, 0.9849 and 0.9872, respectively) exceeded the mean value of pseudogenized branches, we placed their inactivation dates at the base of each clade. We were unable to date *ucp1* inactivation in Pilosa as only remnants of *ucp1* (exon 6) were identified from available extinct Darwin’s ground sloth (*Myiodon*), three-toed sloth (*Bradypus*), two-toed sloth (*Choleopus*) and silky anteater (*Cyclopes didactylus*) genomic sequences; shared inactivating mutations in the latter three species, however, indicate *ucp1* was pseudogenized prior to their radiation ~55.03 Ma.

Transitional branch	Inactivation date (Ma)
<i>Dasyopus novemcinctus</i>	65.55
Pilosa	>55.03
<i>Procavia capensis</i>	33.5–27.3
Proboscidea	42.9–37.5
Sirenia	46.0–44.8
Suidae (<i>Sus spp.</i>)	41.2–36.4
Cetacea	52.75
Equus	25.1–20.7
<i>Manis pentadactyla</i>	74.69

Table S4. Results of binary state speciation and extinction (BiSSE) models with and without constraints on the diversification rate for five trees from Faurby and Svenning (194).

Tree #	Model	Constraints	lambda0	lambda1	mu0	mu1	q01	q10	parameters	Ln Lik	AIC	p value
1	No reversal	q10 = 0	0.2990537	0.2118425	0.2301557	0.1818380	0.0001916	0	5	-14264	28539	2.32E-05
1	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.2985928	0.2985928	0.2333964	0.2333964	0.0001907	0	3	-14275	28556	
2	No reversal	q10 = 0	0.2990537	0.2118425	0.2301557	0.1818380	0.0001916	0	5	-14264	28539	2.32E-05
2	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.2985928	0.2985928	0.2333964	0.2333964	0.0001907	0	3	-14275	28556	
3	No reversal	q10 = 0	0.2990537	0.2118425	0.2301557	0.1818380	0.0001916	0	5	-14264	28539	2.32E-05
3	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.2985928	0.2985928	0.2333964	0.2333964	0.0001907	0	3	-14275	28556	
4	No reversal	q10 = 0	0.3102003	0.1427713	0.2429003	0.0985164	0.0001410	0	5	-14178	28366	9.45E-10
4	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.3041617	0.3041617	0.2393333	0.2393333	0.0001951	0	3	-14199	28404	
5	No reversal	q10 = 0	0.3102003	0.1427713	0.2429003	0.0985164	0.0001410	0	5	-14178	28366	9.45E-10
5	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.3041617	0.3041617	0.2393333	0.2393333	0.0001951	0	3	-14199	28404	

Table S5. Deleterious mutations found in *ucp2* and *ucp3* sequences of placental mammal taxa. Base numbers correspond to position in multiple species alignments (data files 3 and 4). Note that the coding region of several *ucp2* and *ucp3* sequences in GenBank (*ucp2*: Tammar wallaby [ABQO020850797.1], orangutan [ABGA01223176.1, ABGA01223175.1], and thirteen-lined ground squirrel [AAQQ01149310.1]; *ucp3*: Northern white-cheeked gibbon [ADFV01111578.1]) showed signs of disruption (indels), though these were not present in the NCBI sequence read archive and thus likely represent assembly errors.

Gene	Species name	Base number	Inactivating mutation	
<i>ucp2</i>	<i>Dasyopus novemcinctus</i>	1	Mutated initiation codon	
		96	1 bp deletion	
		348-349	2 bp deletion	
		396-397	2 bp deletion	
		463	1 bp deletion	
		646-648	Nonsense mutation	
		653-654	2 bp deletion	
		673	1 bp deletion	
		889	1 bp deletion	
		906	1 bp deletion	
		930	1 bp deletion	
<i>ucp3</i>	<i>Dasyopus novemcinctus</i>	752	1 bp deletion	
		<i>Oryctolagus cuniculus</i>	54	1 bp deletion
			78	1 bp deletion

Table S6. Specimen data and sources of tissue samples used for PCR amplification and DNA hybridization capture

Common name	Scientific name	Sample	Provided by
Xenarthra			
Brown-throated sloth	<i>Bradypus variegatus</i>	Genomic DNA, previously extracted	M. Springer, University of California, Riverside
Silky anteater	<i>Cyclopes didactylus</i>	Genomic DNA, previously extracted (75)	M. Springer, University of California, Riverside
Paenungulata			
Dugong	<i>Dugong dugon</i>	Two genomic DNA samples, Female, Mabuiag Island, Torres Straits, Australia, previously extracted (61); Sample codes: MD33 and MD118	D. McDonald, James Cook University, Australia
Steller's sea cow	<i>Hydrodamalis gigas</i>	Three bone samples housed in the Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russian Federation), previously extracted (62, 63) ZI 6852: Mandible collected from Bering Island, Komandorskii in 1884 ZI6853: Humerus, no collection information ZI 17170: Scapula, no collection information	R. MacPhee, American Museum of Natural History
Asian elephant	<i>Elephas maximus</i>	Genomic DNA, Male, previously extracted (61)	W. Korver, Bowmanville Zoo, Ontario, Canada
African elephant	<i>Loxodonta africana</i>	Genomic DNA, Female, previously extracted (61)	W. Korver, Bowmanville Zoo, Ontario, Canada
Laurasiatheria			
Pygmy hippopotamus	<i>Choeropsis liberiensis</i>	Genomic DNA, previously extracted (64)	New York Zoological Society
Bowhead whale	<i>Balaena mysticetus</i>	Genomic DNA	SWFSC
Beluga whale	<i>Delphinapterus leucas</i>	Genomic DNA, previously extracted (64)	New York Zoological Society
Grant's zebra	<i>Equus quagga boehmi</i>	Skeletal muscle from stillborn calf	S. Petersen, Assiniboine Park Zoo, Manitoba, Canada
Malayan tapir	<i>Tapirus indicus</i>	Blood	M. Bertelsen, Copenhagen Zoo, Frederiksberg, Denmark T. Gilbert and E. Willerslev, University of Copenhagen
Woolly rhinoceros	<i>Coelodonta antiquitatis</i>	Permafrost preserved tooth and bone samples housed at the Centre for GeoGenetics at the Natural History museum of Denmark (Copenhagen, Denmark) CGG_1_000024: Tooth, Bol'shoi Lyakhovsky Island, Novaya Sibir' Island, Siberia, Russia CGG_1_000029: Bone, Badyarikha, Indigirka Lowland, Russia CGG_1_000031: Bone, Khetachan Creek Mine, Kolyma Lowland, Russia	T. Gilbert and E. Willerslev, University of Copenhagen
Black rhinoceros	<i>Diceros bicornis</i>	Genomic DNA, previously extracted	CRES
Sumatran rhinoceros	<i>Dicerorhinus sumatrensis</i>	Blood excised from a leech that fed on a Sumatran rhinoceros	R. Havmøller, T. Gilbert, and E. Willerslev, University of Copenhagen Leibniz-Institute for Zoo and Wildlife Research, Berlin, Germany
Indian rhinoceros	<i>Rhinoceros unicornis</i>	Genomic DNA, previously extracted	CRES

Table S7. Fossil constraints used in timetree analysis.^A

Constrained Clade	Min Age	Max Age	Basis for Max Age	Oldest Fossil	Comments/References
1. Metatheria	65.18	83.8	SB	<i>Peradectes</i> (Puercan); <i>Minoperadectes</i> (Early Wasatchian)	Horovitz <i>et al.</i> (77) reported Puercan <i>Peradectes</i> (age = 65.18 Ma) as the oldest crown metatherian and recovered this taxon as a stem didelphimorphian. Benton <i>et al.</i> (73) cited Horovitz <i>et al.</i> (77) in support of a younger age for Metatheria but failed to describe their rationale for excluding <i>Peradectes</i> from crown Metatheria.
2. Xenarthra	47.8	66.0	PB	<i>Riostegotherium</i>	<i>Riostegotherium</i> is from the Itaboraian (78). The Itaboraian was originally considered equivalent to the Selandian stage (79), but Woodburne <i>et al.</i> (80) suggested an early Eocene age (minimum = 47.8 Ma). The first outgroup to Xenarthra is Afrotheria, Boreoeutheria, or Epitheria, all of which have early Paleocene representatives.
3. Proboscidea (= <i>Loxodonta</i> to <i>Elephas</i>)	6.8	11.62	SB	<i>Loxodonta</i>	Vignaud <i>et al.</i> (81) reported <i>Loxodonta</i> in their faunal list for a hominid locality in Chad. Vignaud <i>et al.</i> 's (81) locality was radiometrically dated by Lebatard <i>et al.</i> (82) to have an age between 6.8 and 7.2 Ma. The oldest <i>Elephas</i> lineage fossils have a minimum age of 5.332 Ma (83, 84).
4. <i>Dugong</i> to <i>Hydrodamalis</i>	28.1	38.0	SB	<i>Crenatosiren</i>	The minimum age is based on the inclusion of early Oligocene taxa (<i>e.g.</i> , <i>Crenatosiren olseni</i>) in crown Dugongidae (63, 85).
5. Sirenia (<i>Dugong</i> to <i>Trichechus</i>)	41.3	59.2	SB	<i>Eotheroides</i>	The minimum age is based on the inclusion of Lutetian age <i>Eotheroides aegyptiacum</i> in crown Sirenia (63, 85-87).
6. Tethytheria	59.2	72.3	SB	<i>Eritherium</i>	<i>Eritherium</i> is a stem proboscidean and is no younger than the late Selandian (88), which has a minimum age of 59.1 Ma.
7. Paenungulata	59.2	72.3	SB	<i>Eritherium</i>	<i>Eritherium</i> is a stem proboscidean and is no younger than the late Selandian (88), which has a minimum age of 59.1 Ma.
8. Chiroptera	47.8	66.0		<i>Dizyia</i>	<i>Dizyia</i> (Phillistidae, Vespertilionoidea) is among the oldest crown chiropterans and is from the Ypresian (89). We used the top of the Ypresian as a minimum age for Yangochiroptera. Phillips (90) suggested a maximum age of 58.9 Ma for Chiroptera. The oldest bats (earliest Eocene, ~55 Ma) were already highly specialized fliers with fully developed wings (91, 92). Bats are one of the least common groups of mammals in the fossil record (89, 91, 93, 94), and in our view it is unnecessarily restrictive to enforce a maximum of 58.9 Ma on crown Chiroptera. Furthermore, molecular studies have rearranged the evolutionary tree for bats and recovered numerous higher-level clades that were previously unrecognized based on morphological data (93, 95, 96). The relationships of several early Eocene fossils (<i>e.g.</i> , <i>Palaeochiropteryx</i> , <i>Archaeonycteris</i> , <i>Hassianonycteris</i>), in turn, lack robust support, and their inclusion or exclusion from crown Chiroptera is sensitive to the enforcement of molecular scaffolds (92, 93, 97). Phylogenetic uncertainties, convergent evolution, and a depauperate fossil record conspire together to limit our understanding of the early evolutionary history of bats and caution against employing a maximum that is younger than the KPg boundary (66 Ma).
9. Megadermatidae to Rhinolophidae+ Hipposideridae	38.0	56	SB	<i>Hipposideros</i>	<i>Hipposideros</i> and <i>Rhinolophus</i> are both known from the Eocene of Europe (98). Eiting and Gunnell (89) indicate that <i>Hipposideros</i> first occurs in the Bartonian whereas <i>Rhinolophus</i> first occurs in the Priabonian.
10. Caniformia (=Canidae to Arctoidea)	38.0	56	SB	<i>Hesperocyon gregarius</i>	<i>Hesperocyon gregarius</i> fossils are known from the Duchesnean (~Bartonian) land mammal age in the middle Eocene (99). <i>H. gregarius</i> is a stem canid (100).
11. Ursidae to other Arctoidea	28.1	41.3	SB	<i>Cephalogale</i>	<i>Cephalogale</i> belongs to the ursid subfamily Hemicyoninae and has emerged as a stem ursid in phylogenetic analyses (101); also see

					Wang <i>et al.</i> (102). <i>Cephalogale</i> is known from the early Oligocene (103). We used the top of the early Oligocene as the minimum for Urside to other arctoids.
12. Musteloidea to Pinnipedia	23.03	38.0	SB/PB/PU	<i>Promartes</i> , <i>Enaliarctos</i>	<i>Mustelavus priscus</i> is first known from the latest Eocene (104). <i>M. priscus</i> is either a stem musteloid (102, 104, 105), and the oldest representative of the crown taxon that includes musteloids and pinnipeds, or a deeper lineage within Caniformes (106). Tomiya's analysis is at odds with molecular studies and recovered <i>Ursus</i> inside of crown Musteloidea. Nevertheless, given the uncertainty regarding <i>Mustelavus</i> based on Tomiya (106), we implemented a minimum of 23.03 Ma for the musteloid-pinniped split based on the oldest musteloid (<i>Promartes</i>) (101, 104) and oldest pinniped (<i>Enaliarctos</i>) (107), both of which are from the late Oligocene.
13. Pinnipedia	20.43	33.9	SB	<i>Desmatophoca brachycephala</i>	<i>Desmatophoca brachycephala</i> is Aquitanian (20.43-23.03 Ma) in age (108). The oldest stem pinnipeds are Chattian (e.g., <i>E. tedfordi</i>). SB yields an older maximum than PB.
14. Carnivora	38.0	66.0	PU	<i>Hesperocyon gregarius</i>	<i>Hesperocyon gregarius</i> is a basal canid in constrained (109) and unconstrained (100, 109) analyses. Viverravids from the early Paleocene are probably stem carnivorans (109), but phylogenetic uncertainty allows for the possibility that they are stem feliforms (110-112). Benton <i>et al.</i> (73) also suggested a maximum of 66 Ma for Carnivora.
15. Perissodactyla	55.5	61.6	SB	<i>Hyracotherium</i>	<i>Hyracotherium</i> first appears in Wa-0 at the beginning of the Eocene (113) and is the oldest crown Perissodactyla.
16. Erinaceidae to Soricidae	61.6	83.8	SB	<i>Adunator</i>	<i>Adunator</i> is the oldest erinaceomorph (114) and is known from the Torrejonian (contained within Danian). The minimum age is 61.6 Ma and the maximum is the base of the Campanian (83.6 +/-0.2 = 83.8 Ma) based on SB.
17. Cetartiodactyla	52.5	66.0	PB/PU	<i>Himalayacetus</i>	<i>Himalayacetus</i> is from shallow benthic zone SB8, which has a minimum age of 52.5 Ma within the Ypresian (47, 114). Mesonychids are a possible sister taxon to Cetartiodactyla, or may even be nested with Cetartiodactyla as the sister to Cetacea (115). The oldest mesonychids are early Paleocene in age and establish a maximum age for Cetartiodactyla.
18. Whippomorpha	52.5	61.6	SB	<i>Himalayacetus</i>	<i>Himalayacetus</i> is from shallow benthic zone SB8, which has a minimum age of 52.5 Ma within the Ypresian (47, 114).
19. Cetacea	34.0	47.8	SB	<i>Llanocetus denticrenatus</i>	<i>Llanocetus</i> is known from the La Meseta Formation of Seymour Island, Antarctica, and is latest Eocene in age (47, 116). <i>Llanocetus</i> is the oldest crown cetacean and a primitive stem mysticete. The oldest odontocetes are stem odontocetes from the Rupelian (early Oligocene) or more precisely from the late early Oligocene (47), although there are also older forms that have not been described. Uhen (47) doesn't provide ages.
20. Mysticeti	20.43	33.9	SB	<i>Morenocetus parvus</i>	<i>Morenocetus parvus</i> is the oldest described species belonging to crown-group Mysticeti, and is from the Aquitanian (minimum age = 20.43 Ma) in the early Miocene of Patagonia (117). There is also an unnamed balaenid (~28 Ma) from the late Oligocene of New Zealand (Kokoamu Greensand) (118), but the phylogenetic position of this taxon is unclear and awaits phylogenetic analysis (J. Geisler, communication to J. Gatesy).
21. Bovinae to Antilopinae	16	28.1	SB	<i>Pseudoeotragus</i>	<i>Pseudoeotragus</i> is a stem antelope and is known from the Burdigalian (73).
22. Haplorrhini (=Tarsiidae to Anthropeidea)	38.0	59.2	PU	Numerous eosimiids	<i>Altitalasius</i> is known from the late Paleocene and is a stem anthropoid in recent cladistic analyses (119, 120), although not in Bloch <i>et al.</i> (121). <i>Anthrasimias</i> (54-55 Ma, Sparnacian) (119, 122) is another putative stem anthropoid, although its anthropoid status has been questioned (120). <i>Algeripithecus</i> is younger (> 45 Ma) and has been referred to Anthropeidea (123). However, recent discoveries of more complete specimens of <i>Algeripithecus</i> suggest that it is not anthropoid (124). Instead, it may belong to the Azibiidae, which are argued to be adapiforms, early euprimates, plesiadapiforms (possible stem

					primates), or even nonprimates (124). As many as 11 species and six genera of Asian eosimiids are known from the middle Eocene of Asia (124). The oldest tarsiid is <i>Tarsius eoacaenus</i> (125, 126) from the middle Eocene of China (minimum age = 38.0 Ma). We used the top of the middle Eocene as a minimum for crown Haplorhini based on the occurrence of numerous eosimiids from the middle Eocene (124). The maximum age allows for the possibility that the late Paleocene <i>Altiatlasius</i> from Morocco is a stem anthropoid (124).
23. Anthropoidea	28.1	56.0	PU	<i>Aegyptopithecus</i>	<i>Aegyptopithecus</i> (early Oligocene~Rupelian) is a stem catarrhine in Kay <i>et al.</i> 's (127) cladistic analyses with molecular scaffolds. The oldest stem platyrrhine is <i>Branisella</i> (127) from the late Oligocene of Bolivia (Salla Beds, Chron 8, age = 25.82-27.02 Ma) (127). Some authors have suggested that <i>Catopithecus browni</i> from the Priabonian is a stem catarrhine, but other authors (<i>e.g.</i> , Williams <i>et al.</i> ; 124) have suggested a stem anthropoid position for this taxon. Seiffert <i>et al.</i> (123) recovered a polytomy with catarrhines, platyrrhines, Parapithecoidea, Proteopithecidae, and <i>Algeripithecus minutus</i> . The age of the latter taxon is Ypresian to basal Lutetian, which established a maximum of 56.0 Ma for the base of Anthropoidea if we allow for phylogenetic uncertainty, <i>i.e.</i> , that <i>Algeripithecus</i> is either a crown anthropoid or one of the first two outgroups. In contrast, Tabuce <i>et al.</i> (128) place <i>Algeripithecus</i> as a stem strepsirrhine based on more complete material.
24. Catarrhini	20.55	38.0	PB/PU	<i>Afropithecus</i>	<i>Afropithecus turkanensis</i> , previously <i>Morotopithecus bishop</i> (129), is dated at 20.6 +/- 0.05 = 20.55 Ma based on overlying lava (130) and is the oldest crown-group catarrhine. It is a stem hominoid (131) or even a crown hominoid (130). The oldest cercopithecoid is <i>Victoriapithecus</i> (132) at ~19 Ma. Oligopithecids (including <i>Catopithecus</i>) are sometimes regarded as stem catarrhines (<i>e.g.</i> , see review of alternate hypotheses in Seiffert and Simons; 133) where <i>Catopithecus</i> is sometimes part of second outgroup to crown catarrhines). The age of <i>Catopithecus</i> is 34.8-33.7 Ma (Chron C13r) based on Seiffert (134), which is mostly within the Priabonian. We used the base of the Priabonian (38.0 Ma) as the maximum age for the base of Catarrhini.
25. Hominoidea	11.608	28.1	SB	<i>Sivapithecus</i>	<i>Sivapithecus</i> is the oldest crown hominoid in Finarelli and Clyde's (131) cladistic and stratocladistic analyses.
26. Strepsirrhini	38.0	56	SB/PB	<i>Saharagalago</i>	<i>Saharagalago misrensis</i> is known from the Bartonian (135) and is assigned to Galagidae; <i>Karanisia</i> is as a crown lorissid in Seiffert <i>et al.</i> 's (135) analysis, although these authors also allow for the possibility that this taxon is a stem lorissid or stem lorissiform. These are the oldest crown strepsirrhines. Adapiforms are paraphyletic at the base of crown Strepsirrhini (136). The oldest adapiforms are early Eocene and establish a maximum age for Strepsirrhini.
27. Chinchillidae to Octodontidae	24.5	38.0	SB/PB	<i>Sallamys</i> , <i>Xylechimys</i> , <i>Deseadomys</i>	Molecular studies (137, 138) suggest that <i>Myocastor</i> , and possibly <i>Capromys</i> , are nested within Echimyidae. The fossil taxa <i>Sallamys</i> , <i>Xylechimys</i> , and <i>Deseadomys</i> (family Echimyidae) are all known from the Deseadean (139), which is mostly late Oligocene and has a minimum age of 24.5 Ma (140, 141). These fossils establish the minimum age for Echimyidae (inclusive of <i>Capromys</i> and <i>Myocastor</i>). <i>Eosallamys</i> , <i>Eoespina</i> , <i>Eosachacui</i> , and <i>Eodelphomys</i> were assigned to Echimyidae and are known from the Santa Rosa Local Fauna of Peru (142, 143). The putative age of this fauna is late Eocene, but this age is based on stage of evolution arguments and remains to be firmly established using more rigorous stratigraphic methods. Hence, we incorporate this information into our maximum age but not our minimum age.
28. Phiomorpha + Caviomorpha	40.94	56	SB/PB	Stem caviomorphs in Antoine <i>et al.</i> (149) including <i>Cachiyacuy</i> and other stem Caviomorpha	This calibration was suggested by Phillips (90) based on fossils that were described by Antoine <i>et al.</i> (144) including <i>Cachiyacuy contamanensis</i> . We retained minimum and maximum ages suggested by Phillips (90). Hartenberger (145) contends that specimens of the early Eocene <i>Glibia</i> are instead the oldest representatives of

					Phiomysidae. Given this possibility we used the base of the early Eocene as the maximum age for Phiomorpha to Cavimorpha.
29. Myomorpha (=Muroidea to Dipodidae)	45	59.2	SB	<i>Pappocricetodon</i>	Eocene deposits in China with <i>Pappocricetodon</i> have been biocorrelated with Bridgerian and early Uintan LMAs of North America (125), and suggest an age of ~45 Ma. SB yields a maximum age of 59.2 Ma for Muroidea + Dipodidae.
30. <i>Mus</i> to <i>Rattus</i>	10.4	15.97		<i>Karmimata</i>	<i>Karmimata darwini</i> is known from the Dhok Pathan Formation, which has an absolute age in the vicinity of 10.4 Ma (73) within the Tortonian stage. <i>K. darwini</i> is closer to <i>Rattus</i> than <i>Mus</i> (73). We used 10.4 Ma as the minimum age for <i>Rattus</i> to <i>Mus</i> following Benton <i>et al.</i> (73). Benton <i>et al.</i> (73) suggested a maximum constraint of 14.0 Ma based on the oldest record of <i>Antemurus</i> , which is found in the Langhian stage. We used the base of the Langhian stage (15.97 Ma) rather than 14.0 Ma for the maximum age of <i>Mus</i> to <i>Rattus</i> .
31. Rodentia	56.0	66.0	SB	<i>Acritoparamys</i> , <i>Paramys</i>	The earliest crown rodents (<i>e.g.</i> , <i>Acritoparamys</i> , <i>Paramys</i>) are late Thanetian in age (73) and establish a minimum age of 56.0 Ma for Rodentia. Given this age, stratigraphic bounding supports a maximum age of 66.0 Ma, which agrees with Benton <i>et al.</i> (73).
32. Lagomorpha	47.6	61.6	SB/PB	Ypresian tarsals	Tarsals from the Vastan mine, India, belong to crown Lagomorpha in phylogenetic analyses (146; p. 1205). The age of the Vastan mine deposits is Ypresian (minimum = 47.6 Ma). The first and second outgroups to crown Lagomorpha are <i>Palaeolagus</i> and <i>Mimotona</i> (147, 148). The latter is from the late Paleocene, which includes the Selandian and Thanetian. <i>Dawsonolagus</i> (149) is another stem lagomorph, but has not been included in cladistic analyses.
33. Mammalia	160.7	227	PB/PU	<i>Ambondro</i>	<i>Ambondro</i> , from the Bathonian, is a crown mammal in a variety of analyses (<i>e.g.</i> , Lou <i>et al.</i> (150) and Woodburne <i>et al.</i> (151); reviewed in Benton <i>et al.</i> (73)), although its affinities with monotremes versus therians remain controversial. We followed Benton <i>et al.</i> (73) and used the top of the Bathonian, <i>i.e.</i> , 166.1 +/- 1.2 Ma = 164.9 Ma. Multituberculata are generally placed inside of crown Mammalia (<i>e.g.</i> , Kielan-Jaworowska <i>et al.</i> ; 152). Haramiyids, in turn, may be related to multituberculates (152-154). The oldest haramiyid is <i>Thomasia</i> from the Norian (155). The oldest mammal fossils are <i>Adelobasileus</i> (155), <i>Gondwanadon</i> (156), and <i>Tikitherium</i> (156) from the Carnian, but there is no suggestion that any of these taxa belong to crown Mammalia. Given the uncertainty associated with the placement of Haramiyidae, including <i>Thomasia</i> , we employed a maximum of 227 Ma (base of Norian).
34. Neognathae	66	86.8	SB	<i>Vegavis iaai</i>	Clarke <i>et al.</i> (157); Benton <i>et al.</i> (73, 114)
35. Amniota	318	332.9	SB	<i>Hylonomus lyelli</i>	<i>Hylonomus</i> is from the Joggins Formation of Nova Scotia. The most recent estimates for the age of the Joggins Formation are in the range of 318-319 Ma (74) (based on Gradstein <i>et al.</i> ; 158). The maximum age is based on the absence of crown amniotes in well-sampled, fossiliferous deposits that occur below the strata with <i>Hylonomus</i> ⁶⁹ .
36. Archosauria to Lepidosauria	255.9	295.9	SB	<i>Protorasaurus speneri</i>	<i>Protorasaurus speneri</i> is the oldest archosauromorph and has a minimum age estimate of 255.9 Ma (73, 114), which falls within the Wuchiapingian stage of the Permian. Benton <i>et al.</i> (73) suggested a maximum age of 295.9 Ma.
37. Tetrapoda	337	351	SB	<i>Lethiscus stocki</i>	<i>Lethiscus</i> is the oldest reptilomorph (stem amniote) and is from the Wardie Shales, which belong to the Lower Oil Shale Group. The age of the Wardie Shales is ~343.5-337 Ma (73) (based on Gradstein <i>et al.</i> ; 158). The maximum age for Tetrapoda is from Benton <i>et al.</i> (73).

^ATable modified and updated after Meredith *et al.* (75). Abbreviations: PB, phylogenetic bracketing; PU, phylogenetic uncertainty; SB, stratigraphic bounding; Ma, million years.

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