www.sciencemag.org/cgi/content/full/340/6138/1234192/suppl/DC1

Supplementary Materials for

Evolution of Mammalian Diving Capacity Traced by Myoglobin Net Surface Charge

Scott Mirceta, Anthony V. Signore, Jennifer M. Burns, Andrew R. Cossins, Kevin L. Campbell, Michael Berenbrink*

*Corresponding author. E-mail: michaelb@liv.ac.uk

Published 14 June 2013, *Science* **340**, 1234192 (2013)
DOI: 10.1126/science.1234192

This PDF file includes:

Supplementary Text

Phylogenetic relationships and divergence times for the composite mammalian tree (Fig. S9) are generally based on Meredith *et al.* (*41*), with the following additions and exceptions:

Paenungulata

The relationship between Sirenia, Hyracoidea and Proboscidea has been notoriously difficult to resolve but they are generally thought to have diverged from each other within a short time period that has been dated to between 64.3 and 62.6 Mya (*41, 64, 65*). Here we accept a Sirenia and Proboscidea sister group relationship (*38, 66*), assigning the time of their split from Hyracoidea to 64.3 Mya and the time of their split from each other to 62.6 Mya. The split between dugong and Steller's sea cow is dated to 22 Mya (*67*). Proboscidean phylogeny and divergence times are based on Rohland *et al.* (*68*).

Eulipotyphla

The Erinacaeidae – Soricidae sister group relationship (*69*), as also revealed in the DNA tree of Meredith *et al.* (*41*), is accepted here. Within Soricidae, a divergence time of 17.0 Mya was used for the *Sorex* - *Blarina* split and a divergence time of 13.9 Mya for the North American and Eurasian *Sorex* species (*S. palustris* and *S. araneus*, respectively) (*70*). Within Talpidae, *Condylura* is recognised as the sister group to Desmaninae + Talpinae (*71*). The fossil record of *Condylura* is sparse, with a humerus tentatively assigned to this genus dating back to 7 – 8 Mya (*72*). Thus, the time of divergence between and Desmaninae + Talpinae has been set at the minimum age of the latter group of 37 Mya (*73*).

Carnivora

Interrelationships of Pinnipedia, Musteloidea and Ursidae follows the nucleotide data set in Meredith *et al.* (*41*) and divergence times and phylogenetic arrangements within these groups follow Higdon *et al.* (*74*), Sato *et al.* (*75*) and Krause *et al*. (*76*), respectively. Phylogeny of Canidae follows Bardeleben *et al*. (*77*). The Cape fox (*Vulpes*) – bat eared fox (*Otocyon*) split is put at 8 Mya, based on the red fox - Arctic fox split (*78*). The African hunting dog (*Lyacon*) – domestic dog (*Canis*) divergence has been put at 5.3 Mya, the age of the oldest *Canis* fossil (*79*). The (*Vulpes*, *Otocyon*) – (*Lycaon*, *Canis*) split at 11.7 Mya is based on the *Cuona* – (*Vulpes*, *Nycteruetes*) split (*80*).

Equidae

The time of the horse – zebra split was taken as 2.4 Mya (*81*).

Ruminantia

Phylogeny and divergence times of Ruminantia follow Fernandez & Vrba (*82*).

Cetacea

Intra relationships and divergence times of this group follows McGowen *et al*. (*83*).

Lagomorpha

The American pika - black lipped pika split was assumed at 10.0 Mya (*84*).

Muridae

Within Muridae, the *Mus* – *Rattus* split was dated to 12.3 Mya (*85*).

Cricetidae

Within Cricetidae, the Arvicolinae (*Ondatra*) – Cricetinae (*Cricetulus*) split was dated at 17.9 Mya (*86*).

Spalacidae

The divergence time of *Spalax carmeli* and *Spalax judaei* within the *S. Ehrenbergi* super species complex was set at 0.2 Mya (*87*).

Primates

In general, primate phylogeny and divergence times were taken from Chatterjee *et al.* (*88*), apart from the New World monkeys (Haplorrhinii), where Opazo *et al.* (*89*) – supported by Wildman *et al.* (*90*) - was followed.

Composite, time-calibrated phylogeny used in this work in Newick tree format

This tree includes Ostrich and Anolis lizard as out group. See Table S1 for scientific species names and Fig. S6 for a tree image.

(Anolis_lizard:270.70000000,Ostrich:270.70000000,((Echidna:36.70000000,Platypus:36.70000000):181.10000000,(((Red_kangaroo:74.70000000,Tasmanian_devil:74.70000000):7.10000000,Opossum:81.80000000):108.20000000,((((Aardvark:78.60000000,(Golden_mole:77.30000000,Elephant_shrew:77.30000000):1.30000000):2.30000000,(Cape_hyrax:64.30000000,((Florida_manatee*:31.40000000,(Dugong*:22.00000000,'Steller''s sea cow*':22.00000000):9.40000000):31.20000000,(African_elephant*:7.60000000,(Asian_elephant*:6.70000000,Woolly_mammoth*:6.70000000):0.90000000):55.00000000):1.70000000):16.60000000):17.80000000,Armadillo:98.70000000):2.60000000,((((((American_water_shrew*:13.90000000,Eurasian_shrew:13.90000000):3.10000000,Short_tailed_shrew*:17.00000000):54.90000000,Hedgehog:71.90000000):3.10000000,(Star-nosed_mole*:37.00000000,Coast_mole*:37.00000000):38.00000000):9.60000000,(('David''s Myotis':66.50000000,Black_flying_fox:66.50000000):15.52000000,((((((((((((Grey_seal:2.10000000,Baikal_seal:2.10000000):0.10000000,Harbour_seal:2.20000000):0.20000000,Ringed_seal*:2.40000000):4.00000000,Harp_seal*:6.40000000):1.60000000,Hooded_seal*:8.00000000):5.00000000,Bearded_seal*:13.00000000):3.00000000,(Weddell_seal*:10.00000000,Elephant_seal*:10.00000000):6.00000000):7.00000000,(((((California_sealion*:0.00000000,Steller_sealion*:0.00000000):6.10000000,Australian_sealion*:6.10000000):2.10000000,Northern_fur_seal*:8.20000000):9.80000000,Walrus*:18.00000000):5.00000000):16.00000000,(Raccoon*:28.40000000,(Badger:12.90000000,(American_mink*:9.60000000,Eurasian_river_otter:9.60000000):3.30000000):15.50000000):10.60000000):0.30000000,((Black_bear:5.05000000,Polar_bear*:5.05000000):14.04000000,Giant_Panda:19.09000000):20.21000000):7.20000000,((Cape_fox:8.00000000,Bat_eared_fox:8.00000000):3.70000000,(African_hunting_dog:5.30000000,dog:5.30000000):6.40000000):34.80000000):8.20000000,Cat:54.70000000):27.28000000,(((Horse:2.40000000,Zebra:2.40000000):58.60000000,White_rhinoceros:61.00000000):17.30000000,(Pig:63.00000000,(((((Cattle:5.80000000,Yak:5.80000000):11.10000000,Water_buffalo:16.90000000):8.50000000,((Goat:11.30000000,Sheep:11.30000000):6.50000000,Tibetan_antelope:17.80000000):7.60000000):6.60000000,Red_deer:32.00000000):25.60000000,(Pygmy_hippo*:53.70000000,((((((Fin_back_whale:7.06000000,Humpback_whale:7.06000000):1.98000000,Grey_whale:9.04000000):1.17000000,('Pygmy Bryde''s whale':3.31000000,Sei_whale:3.31000000):6.90000000):3.59000000,Minke_whale:13.80000000):14.99000000,Bowhead*:28.79000000):7.57000000,(((Pygmy_sperm_whale:9.33000000,Dwarf_sperm_whale:9.33000000):14.88000000,Sperm_whale:24.21000000):10.48000000,((((((('Stejneger''s beaked whale':13.48000000,'Hubbs'' beaked whale':13.48000000):0.96000000,'Sowerby''s beaked whale*':14.44000000):1.47000000,'Longman''s beaked whale':15.91000000):1.20000000,Northern_bottlenose_whale*:17.11000000):1.46000000,'Cuvier''s beaked whale':18.57000000):12.65000000,(Amazon_river_dolphin:24.75000000,((Narwhal*:15.54000000,(Harbor_porpoise:3.75000000,'Dall''s porpoise':3.75000000):11.79000000):3.12000000,(Killer_whale:10.08000000,(White_beaked_dolphin*:8.85000000,(('Risso''s dolphin*':4.20000000,(Long_fin_pilot_whale:2.12000000,Melon_head_whale:2.12000000):2.08000000):4.16000000,(Bridled_dolphin:3.51000000,(Saddleback_dolphin:2.18000000,Bottlenose_dolphin:2.18000000):1.33000000):4.85000000):0.49000000):1.23000000):8.58000000):6.09000000):6.47000000):3.47000000):1.67000000):17.34000000):3.90000000):5.40000000):17.70000000):1.28000000):0.04000000):2.58000000):7.40000000,((((Rabbit:50.20000000,(Black_lipped_pika:10.00000000,American_pika:10.00000000):40.20000000):29.30000000,((Squirrel*:67.40000000,(Gundi:61.10000000,(Naked_mole_rat:47.20000000,((Viscacha:38.90000000,Casiragua:38.90000000):3.10000000,Guinea_pig:42.00000000):5.2000000):13.90000000):6.30000000):1.60000000,((Kangaroo_rat:58.80000000,Eurasian_beaver:58.80000000):6.30000000,((Mt._Carmel_mole_rat:0.20000000,'Ehrenberg''s mole rat':0.20000000):44.70000000,((House_mouse:12.30000000,Norway_rat:12.30000000):13.60000000,(Chinese_hamster:17.90000000,Muskrat:17.9000000):8.00000000):19.00000000):20.20000000):3.90000000):10.50000000):3.30000000,Tree_Shrew:82.80000000):0.50000000,((((Potto:31.80000000,Thick_Tailed_Bush_Baby:31.80000000):5.70000000,Slow_Loris:37.50000000):14.10000000,(Grey_mouse_Lemur:32.40000000,Weasel_Lemur:32.40000000):19.20000000):19.90000000,((Common_Woolly_Monkey:26.00000000,(White_tuft_ear_marmoset:22.75000000,(Douroucouli:22.05000000,(Squirrel_monkey:19.50000000,Brown_capuchin:19.50000000):2.55000000):0.70000000):3.25000000):16.80000000,((Hanuman_langur:23.40000000,(Red_guenon:18.60000000,(Olive_baboon:17.10000000,(Rhesus_monkey:7.40000000,Crab_eating_macaque:7.40000000):9.70000000):1.50000000):4.80000000):5.9000

0000,((Siamang:10.30000000,(White_cheeked_gibbon:9.20000000,Agile_gibbon:9.20000000):1.10000000):11.200
00000,(Orangutan:15.90000000,(Mountain_gorilla:10.70000000,(Chimpanzee:8.10000000,Human:8.10000000):2.6
0000000):5.20000000):5.60000000):7.80000000):13.50000000):28.70000000):11.80000000):8.70000000):9.30000
000):88.70000000):27.80000000):145.50000000);

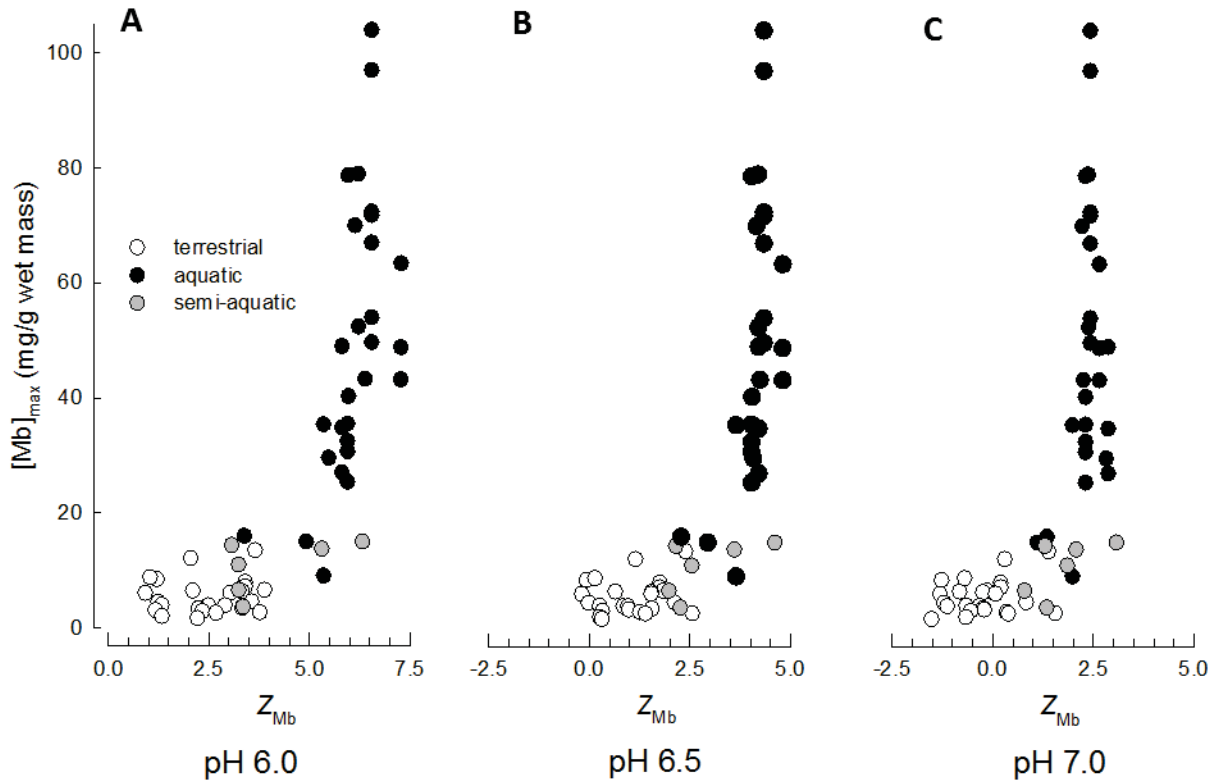
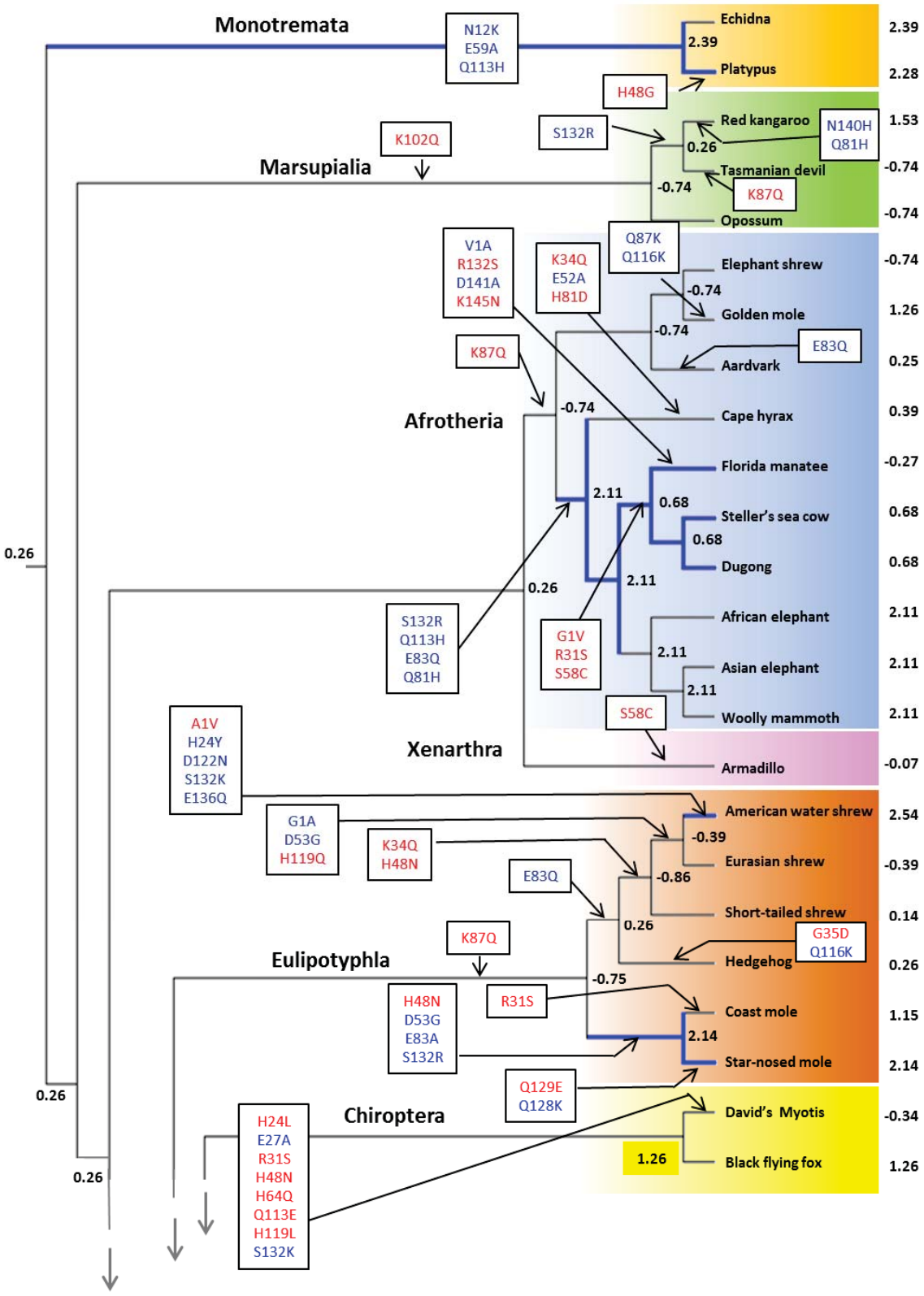


Fig. S1.

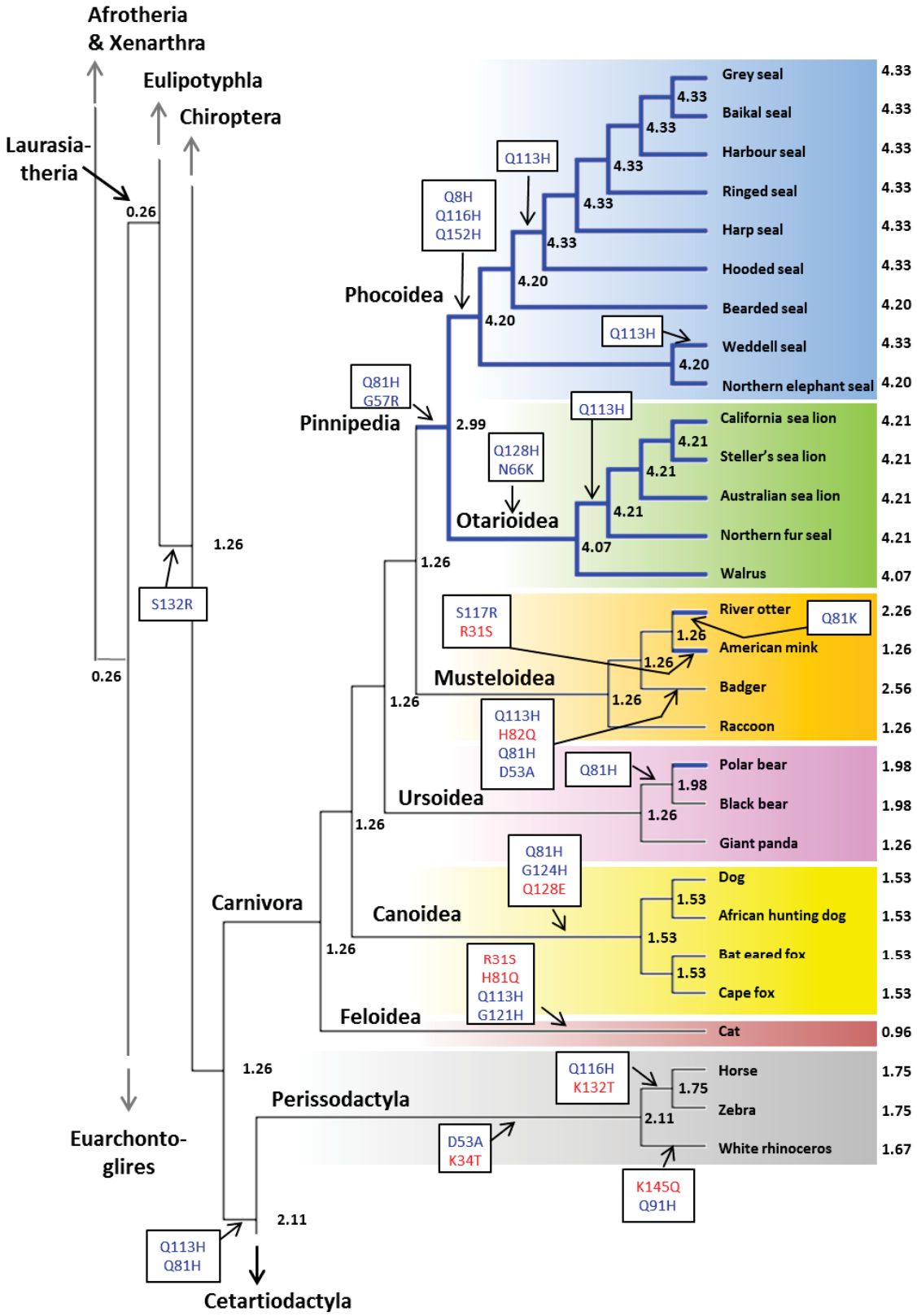
Mb net surface charge, Z_{Mb}, and maximal muscle concentration, $[Mb]_{max}$, in terrestrial, semi-aquatic and aquatic mammals. Z_{Mb} was obtained by modeling for a) pH 6.0, b) pH 6.5 and c) pH7.0 (*17*).

(Fig. S2)



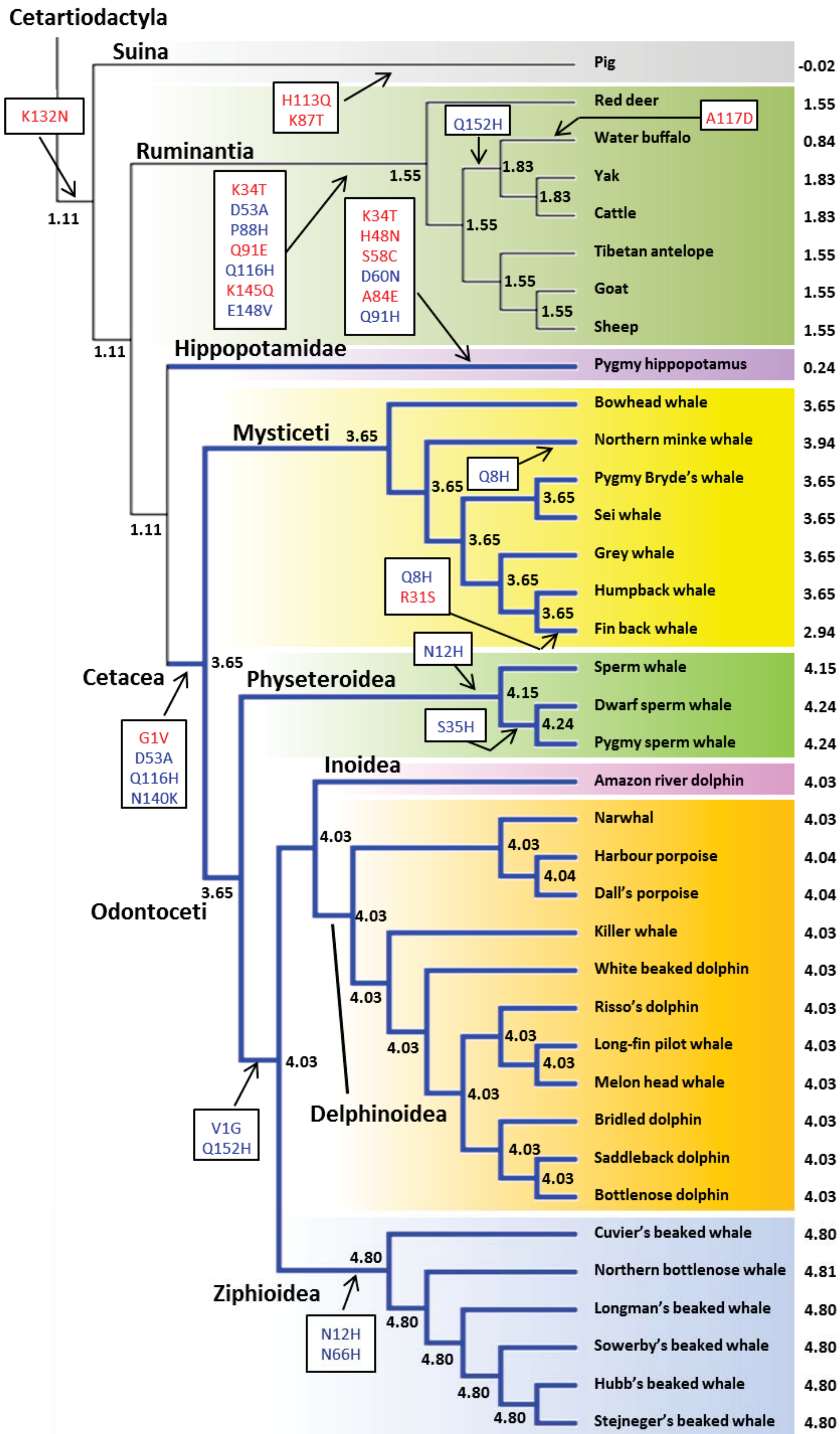
(continues)

(Fig. S2 continued)



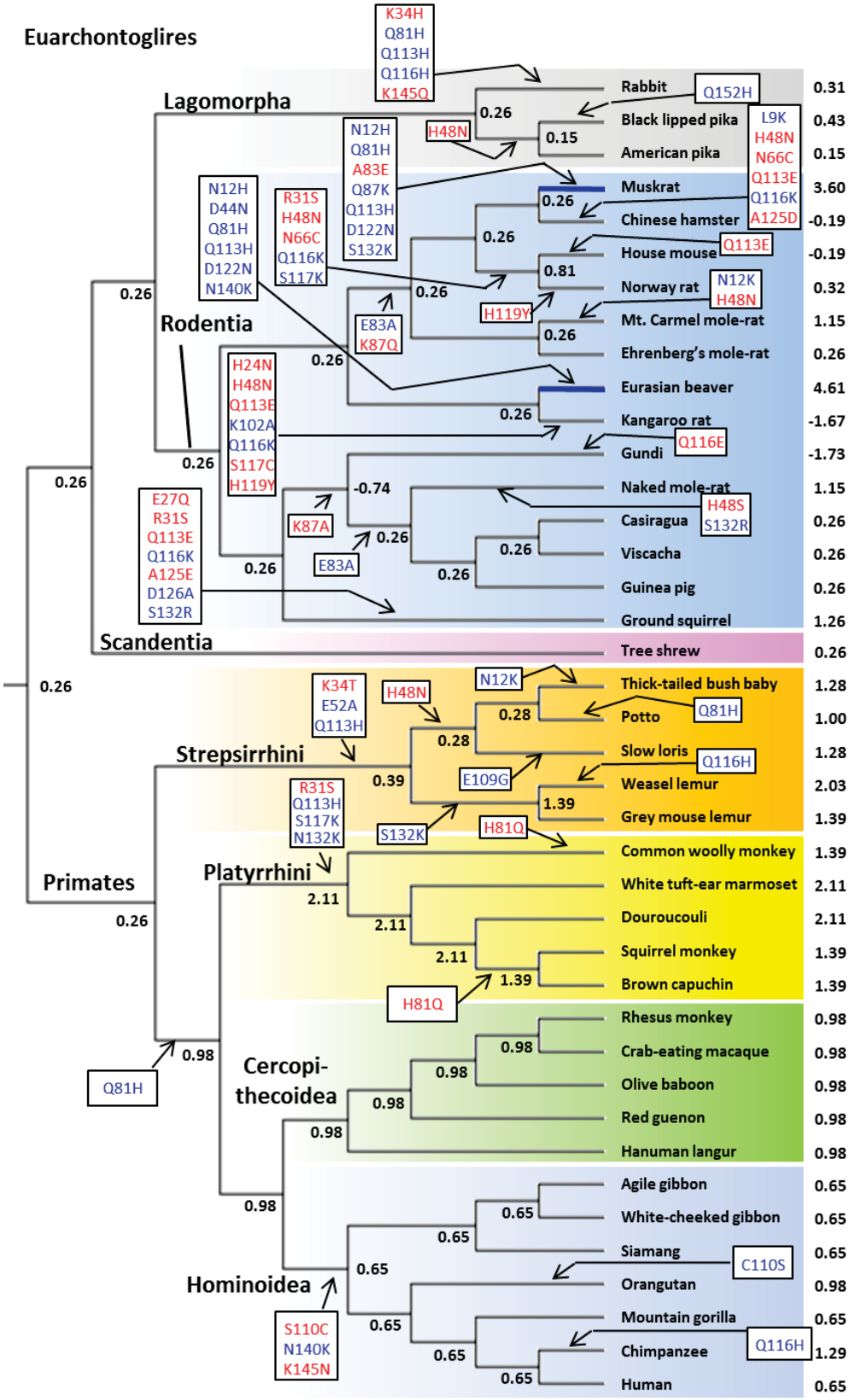
(continues)

(Fig. S2 continued)



(continues)

(Fig. S2 continued)



(continues)

(Fig. S2 continued)

Fig. S2

Evolutionary reconstruction of Mb net surface charge (Z_{Mb}) in mammals. Z_{Mb} (black numbers) was obtained by modeling Mb primary structure onto the tertiary structure of the protein and using site specific, conserved ionization constants (*17*). Ancestral primary structure was reconstructed by Maximum Likelihood on a composite mammalian phylogeny from the primary Mb sequences in recent mammals, using the Dayhoff+G model of sequence evolution in MEGA5. Values after species names and at nodes are Z_{Mb} values at pH 6.5 in recent species and their ancestors, respectively. Text boxes and arrows indicate all sites and branches along which charge-increasing (blue font) and charge-decreasing (red font) amino acid substitutions have been reconstructed. Blue tree branches indicate semi-aquatic or aquatic lineages.

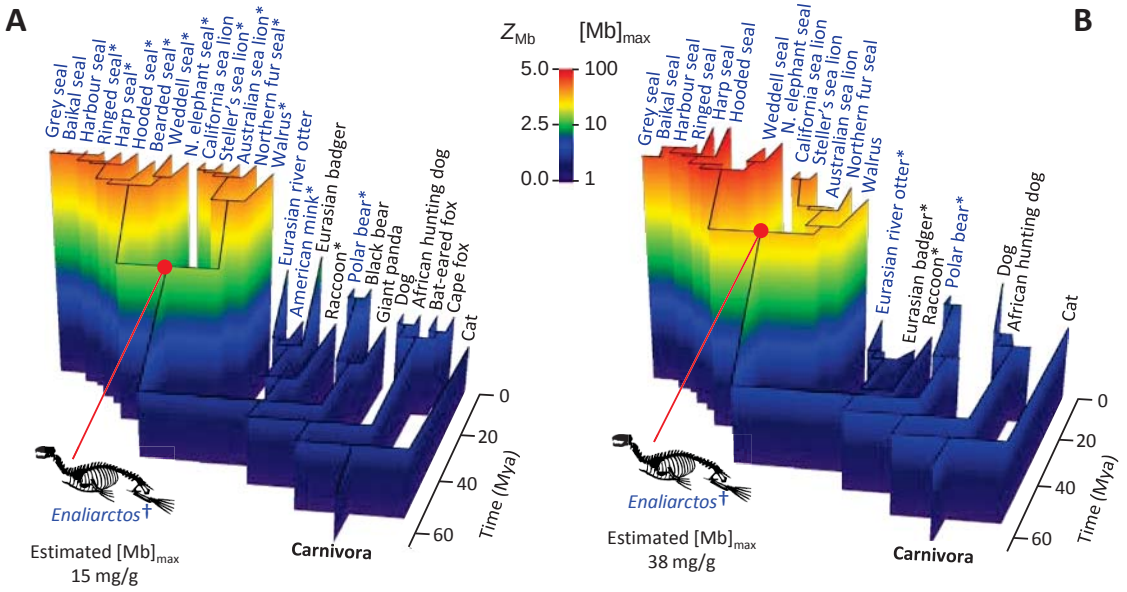
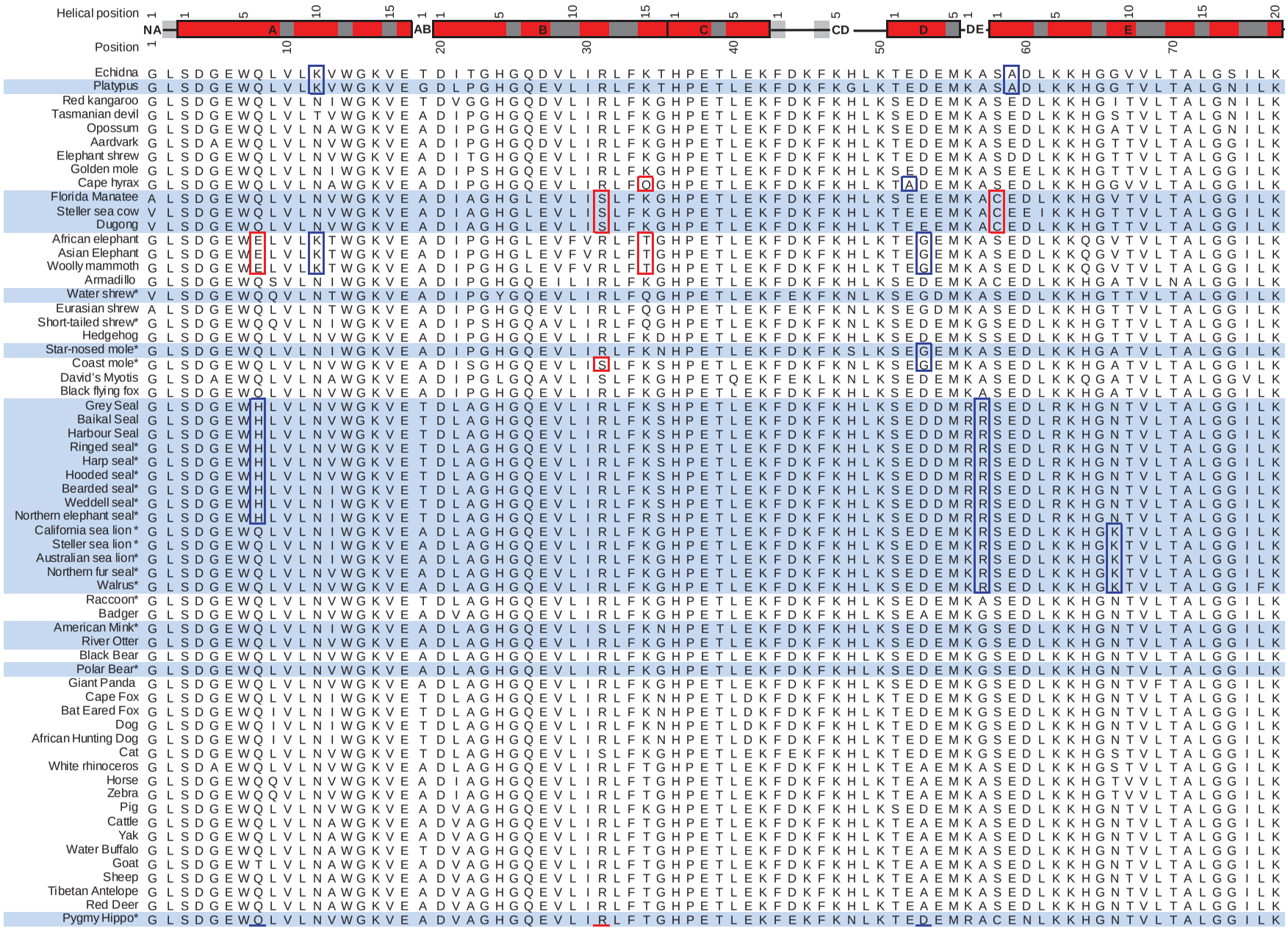


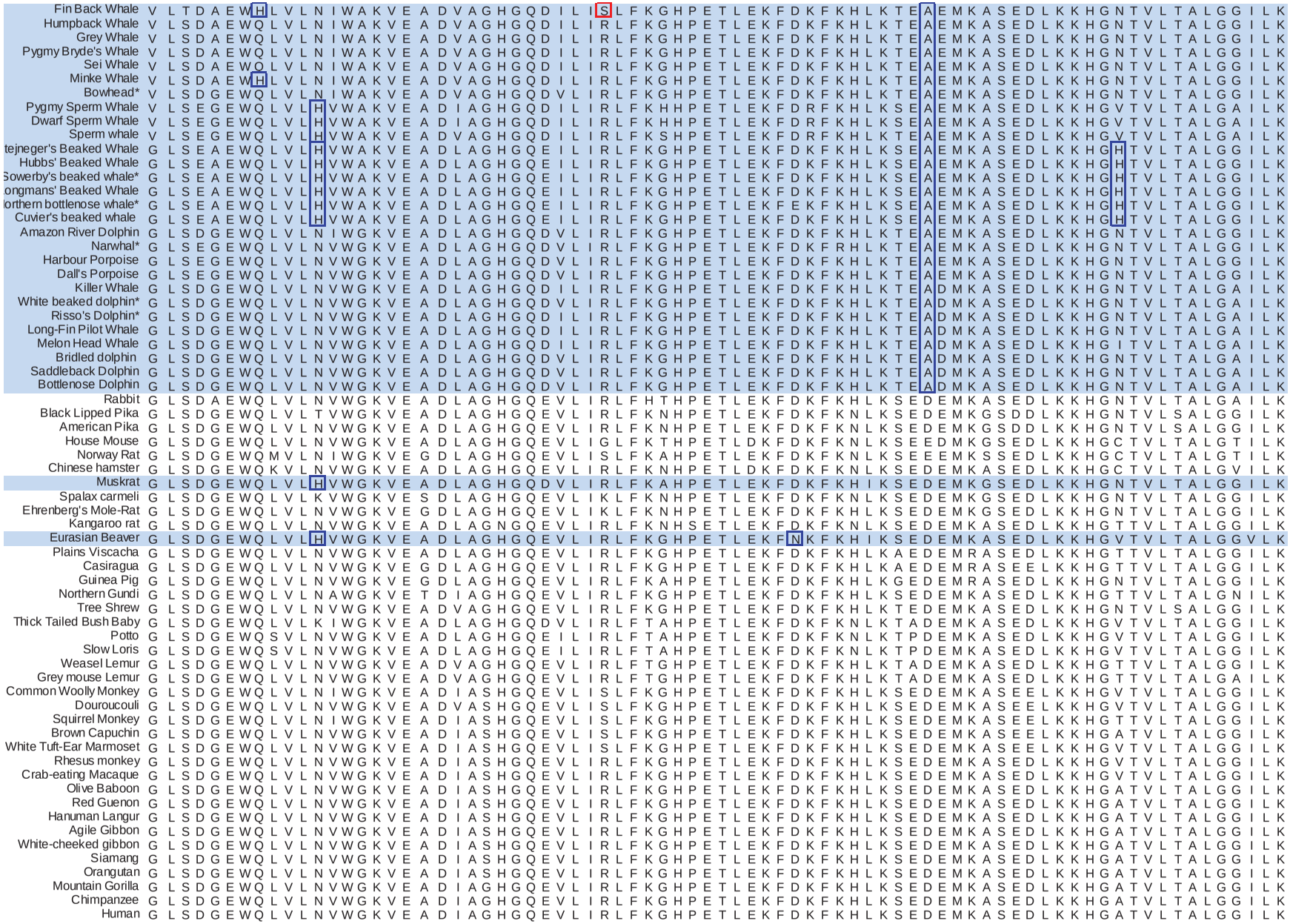
Fig. S3

Comparison of using evolutionary reconstruction of myoglobin net surface charge (Z_{Mb}; A) or maximum parsimony (MP) reconstruction of values measured in extant species (B) for estimating evolution of maximum myoglobin muscle concentration ($[Mb]_{max}$) in Carnivora. The MP method fails to resolve the convergent nature of $[Mb]_{max}$ increases during pinniped evolution and leads to a more than two-fold higher estimate of $[Mb]_{max}$ in the stem pinniped *Enaliarctos*. Species for which no $[Mb]_{max}$ value is available are pruned from the tree in panel *B*. *Mb sequences (A) or $[Mb]_{max}$ values (B) determined in the present study.

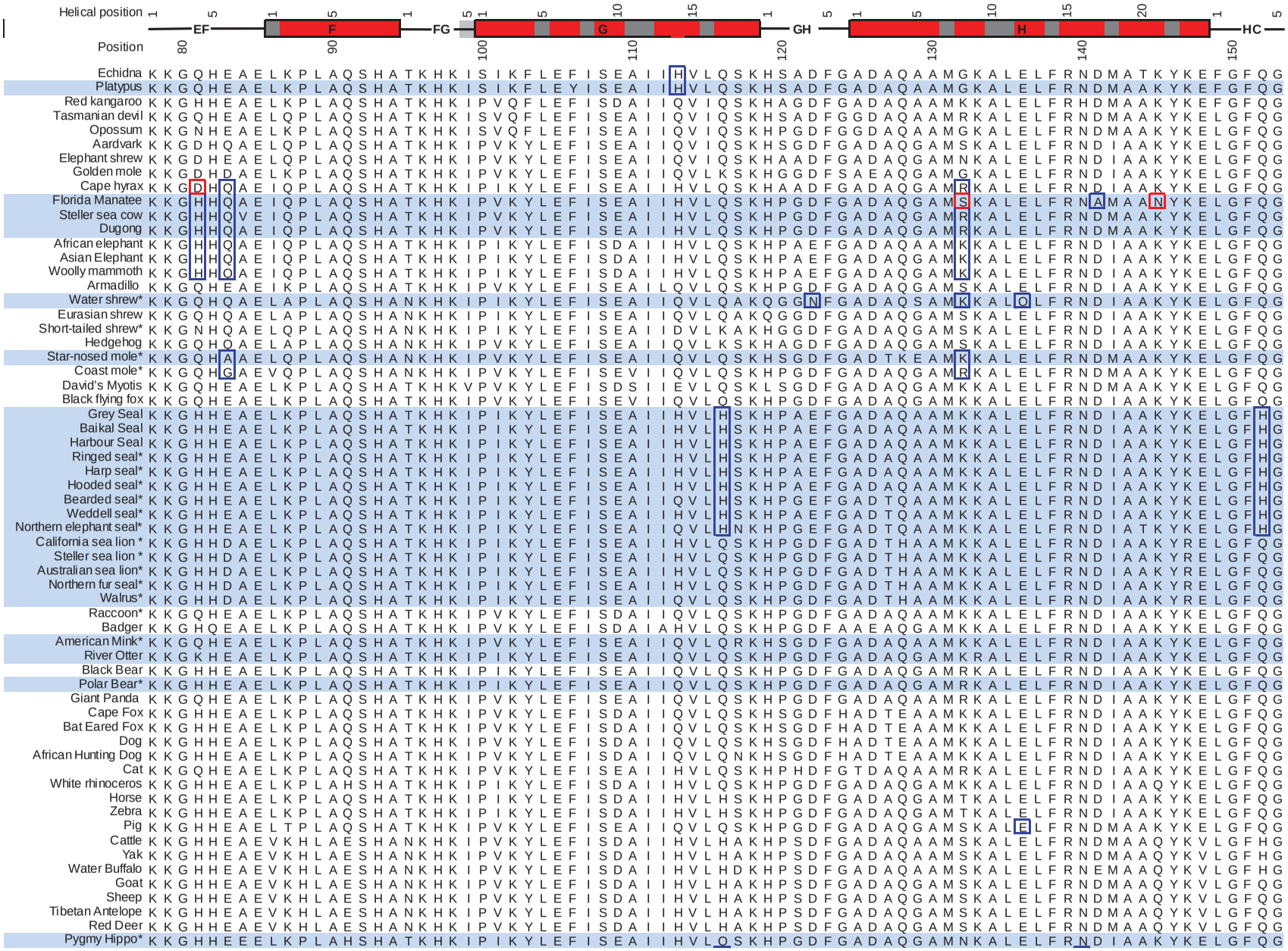
A (Fig. S4)


Helical position
NA
A
AB
B
C
CD
D
DE
E
Position
Echidna
Platypus
Red kangaroo
Tasmanian devil
Opossum
Aardvark
Elephant shrew
Golden mole
Cape hyrax
Florida Manatee
Steller sea cow
Dugong
African elephant
Asian Elephant
Woolly mammoth
Armadillo
Water shrew*
Eurasian shrew
Short-tailed shrew*
Hedgehog
Star-nosed mole*
Coast mole*
David's Myotis
Black flying fox
Grey Seal
Baikal Seal
Harbour Seal
Ringed seal*
Harp seal*
Hooded seal*
Bearded seal*
Weddell seal*
Northern elephant seal*
California sea lion *
Steller sea lion *
Australian sea lion*
Northern fur seal*
Walrus*
Raccoon*
Badger
American Mink*
River Otter
Black Bear
Polar Bear*
Giant Panda
Cape Fox
Bat Eared Fox
Dog
African Hunting Dog
Cat
White rhinoceros
Horse
Zebra
Pig
Cattle
Yak
Water Buffalo
Goat
Sheep
Tibetan Antelope
Red Deer
Pygmy Hippo*

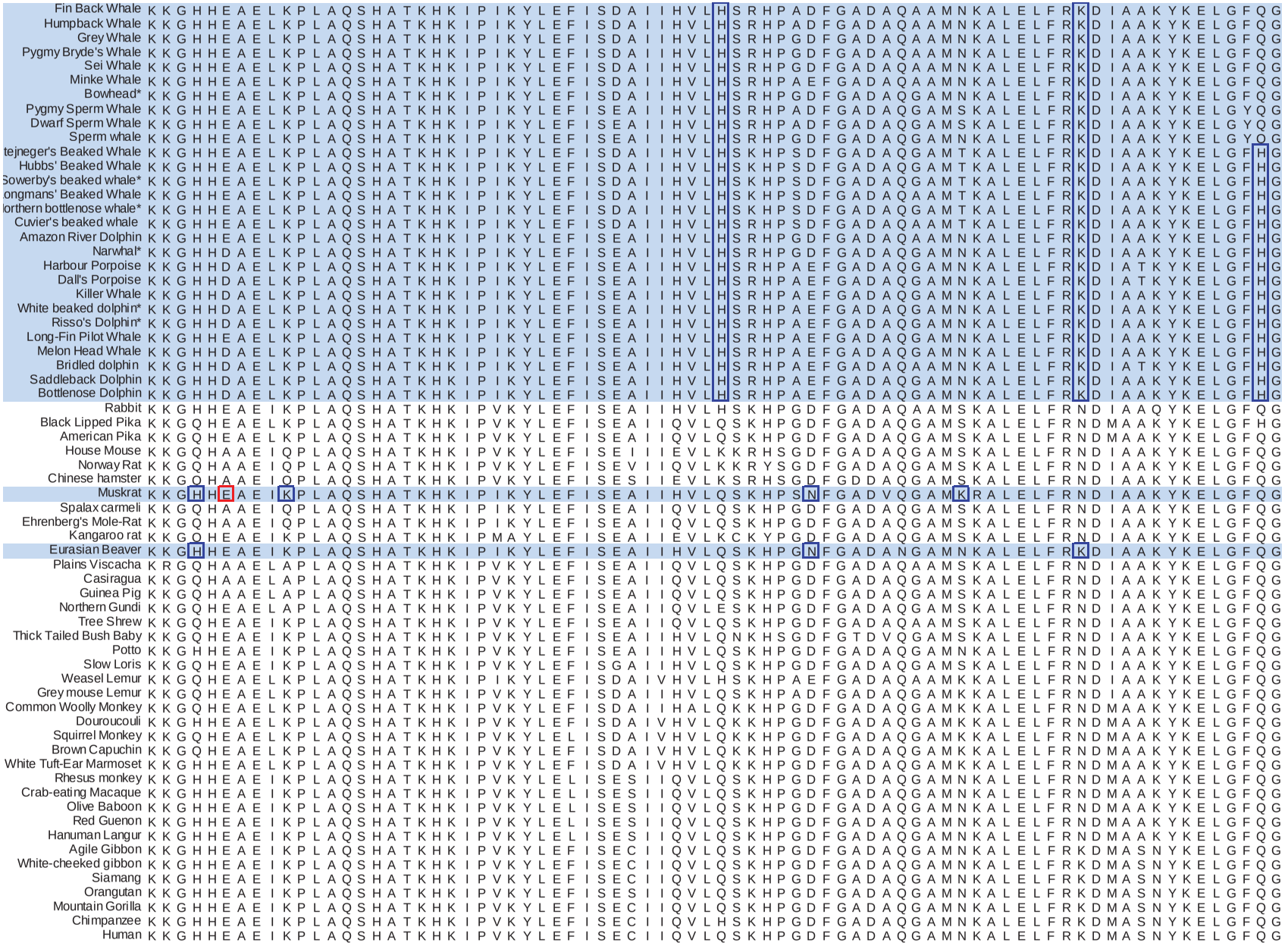
B (Fig. S4)


Fin Back Whale V L T D A E W H L V L N I W A K V E A D V A G H G Q D I L I S L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Humpback Whale V L S D A E W Q L V L N I W A K V E A D V A G H G Q D I L I R L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Grey Whale V L S D A E W Q L V L N I W A K V E A D V A G H G Q D I L I R L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Pygmy Bryde's Whale V L S D A E W Q L V L N I W A K V E A D V A G H G Q D I L I R L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Sei Whale V L S D A E W Q L V L N I W A K V E A D V A G H G Q D I L I R L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Minke Whale V L S D A E W H L V L N I W A K V E A D V A G H G Q D I L I R L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Bowhead* V L S D G E W Q L V L N I W A K V E A D V A G H G Q D V L I R L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Pygmy Sperm Whale V L S E G E W Q L V L H V W A K V E A D I A G H G Q D I L I R L F K H H P E T L E K F D R F K H L K S E A E M K A S E D L K K H G V T V L T A L G A I L K
Dwarf Sperm Whale V L S E G E W Q L V L H V W A K V E A D I A G H G Q D I L I R L F K H H P E T L E K F D R F K H L K S E A E M K A S E D L K K H G V T V L T A L G A I L K
Sperm whale V L S E G E W Q L V L H V W A K V E A D V A G H G Q D I L I R L F K S H P E T L E K F D R F K H L K T E A E M K A S E D L K K H G V T V L T A L G A I L K
tejneger's Beaked Whale G L S E A E W Q L V L H V W A K V E A D L A G H G Q E I L I R L F K G H P E T L E K F D K F K H L K S E A E M K A S E D L K K H G H T V L T A L G G I L K
Hubbs' Beaked Whale G L S E A E W Q L V L H V W A K V E A D L A G H G Q E I L I R L F K G H P E T L E K F D K F K H L K S E A E M K A S E D L K K H G H T V L T A L G G I L K
Sowerby's beaked whale* G L S E A E W Q L V L H V W A K V E A D L A G H G Q E I L I R L F K G H P E T L E K F D K F K H L K S E A E M K A S E D L K K H G H T V L T A L G G I L K
ongmans' Beaked Whale G L S E A E W Q L V L H V W A K V E A D L A G H G Q E I L I R L F K G H P E T L E K F D K F K H L K S E A E M K A S E D L K K H G H T V L T A L G G I L K
lorthern bottlenose whale* G L S E A E W Q L V L H V W A K V E A D L A G H G Q E I L I R L F K G H P E T L E K F E K F K H L K S E A E M K A S E D L K K H G H T V L T A L G G I L K
Cuvier's beaked whale G L S E A E W Q L V L H V W A K V E A D L A G H G Q E I L I R L F K G H P E T L E K F D K F K H L K S E A E M K A S E D L K K H G H T V L T A L G G I L K
Amazon River Dolphin G L S D G E W Q L V L N I W G K V E A D L A G H G Q D V L I R L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Narwhal* G L S E G E W Q L V L N V W G K V E A D L A G H G Q D V L I R L F K G H P E T L E K F D K F R H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Harbour Porpoise G L S E G E W Q L V L N V W G K V E A D L A G H G Q D V L I R L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Dall's Porpoise G L S E G E W Q L V L N V W G K V E A D L A G H G Q D V L I R L F K G H P E T L E K F D K F K H L K T E A E M K A S E D L K K H G N T V L T A L G G I L K
Killer Whale G L S D G E W Q L V L N V W G K V E A D L A G H G Q D I L I R L F K G H P E T L E K F D K F K H L K T E A D M K A S E D L K K H G N T V L T A L G A I L K
White beaked dolphin* G L S D G E W Q L V L N V W G K V E A D L A G H G Q D V L I R L F K G H P E T L E K F D K F K H L K T E A D M K A S E D L K K H G N T V L T A L G A I L K
Risso's Dolphin* G L S D G E W Q L V L N V W G K V E A D L A G H G Q D I L I R L F K G H P E T L E K F D K F K H L K T E A D M K A S E D L K K H G N T V L T A L G A I L K
Long-Fin Pilot Whale G L S D G E W Q L V L N V W G K V E A D L A G H G Q D I L I R L F K G H P E T L E K F D K F K H L K T E A D M K A S E D L K K H G N T V L T A L G A I L K
Melon Head Whale G L S D G E W Q L V L N V W G K V E A D L A G H G Q D I L I R L F K G H P E T L E K F D K F K H L K T E A D M K A S E D L K K H G I T V L T A L G A I L K
Bridled dolphin G L S D G E W Q L V L N V W G K V E A D L A G H G Q D V L I R L F K G H P E T L E K F D K F K H L K T E A D M K A S E D L K K H G N T V L T A L G A I L K
Saddleback Dolphin G L S D G E W Q L V L N V W G K V E A D L A G H G Q D V L I R L F K G H P E T L E K F D K F K H L K T E A D M K A S E D L K K H G N T V L T A L G A I L K
Bottlenose Dolphin G L S D G E W Q L V L N V W G K V E A D L A G H G Q D V L I R L F K G H P E T L E K F D K F K H L K T E A D M K A S E D L K K H G N T V L T A L G A I L K
Rabbit G L S D A E W Q L V L N V W G K V E A D L A G H G Q E V L I R L F H T H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G N T V L T A L G A I L K
Black Lipped Pika G L S D G E W Q L V L T V W G K V E A D L A G H G Q E V L I R L F K N H P E T L E K F D K F K N L K S E D E M K G S D D L K K H G N T V L S A L G G I L K
American Pika G L S D G E W Q L V L N V W G K V E A D L A G H G Q E V L I R L F K N H P E T L E K F D K F K N L K S E D E M K G S D D L K K H G N T V L S A L G G I L K
House Mouse G L S D G E W Q L V L N V W G K V E A D L A G H G Q E V L I G L F K T H P E T L D K F D K F K N L K S E E D M K G S E D L K K H G C T V L T A L G T I L K
Norway Rat G L S D G E W Q M V L N I W G K V E G D L A G H G Q E V L I S L F K A H P E T L E K F D K F K N L K S E E E M K S S E D L K K H G C T V L T A L G T I L K
Chinese hamster G L S D G E W Q K V L N V W G K V E A D L A G H G Q E V L I R L F K N H P E T L D K F D K F K N L K S E D E M K A S E D L K K H G C T V L T A L G V I L K
Muskrat G L S D G E W Q L V L H V W G K V E A D L A G H G Q D V L I R L F K A H P E T L E K F D K F K H I K S E D E M K G S E D L K K H G N T V L T A L G G I L K
Spalax carmeli G L S D G E W Q L V L K V W G K V E S D L A G H G Q E V L I K L F K N H P E T L E K F D K F K N L K S E D E M K G S E D L K K H G N T V L T A L G G I L K
Ehrenberg's Mole-Rat G L S D G E W Q L V L N V W G K V E G D L A G H G Q E V L I K L F K N H P E T L E K F D K F K H L K S E D E M K G S E D L K K H G N T V L T A L G G I L K
Kangaroo rat G L S D G E W Q L V L N V W G K V E A D L A G N G Q E V L I R L F K N H S E T L E K F D K F K N L K S E D E M K A S E D L K K H G T T V L T A L G G I L K
Eurasian Beaver G L S D G E W Q L V L H V W G K V E A D L A G H G Q E V L I R L F K G H P E T L E K F N K F K H I K S E D E M K A S E D L K K H G V T V L T A L G G V L K
Plains Viscacha G L S D G E W Q L V L N V W G K V E A D L A G H G Q E V L I R L F K G H P E T L E K F D K F K H L K A E D E M R A S E D L K K H G T T V L T A L G G I L K
Casiragua G L S D G E W Q L V L N V W G K V E G D L A G H G Q E V L I R L F K G H P E T L E K F D K F K H L K A E D E M R A S E E L K K H G T T V L T A L G G I L K
Guinea Pig G L S D G E W Q L V L N V W G K V E G D L A G H G Q E V L I R L F K A H P E T L E K F D K F K H L K G E D E M R A S E D L K K H G N T V L T A L G G I L K
Northern Gundi G L S D G E W Q L V L N A W G K V E T D I A G H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G T T V L T A L G N I L K
Tree Shrew G L S D G E W Q L V L N V W G K V E A D V A G H G Q E V L I R L F K G H P E T L E K F D K F K H L K T E D E M K A S E D L K K H G N T V L S A L G G I L K
Thick Tailed Bush Baby G L S D G E W Q L V L K I W G K V E A D L A G H G Q D V L I R L F T A H P E T L E K F D K F K N L K T A D E M K A S E D L K K H G V T V L T A L G G I L K
Potto G L S D G E W Q S V L N V W G K V E A D L A G H G Q E I L I R L F T A H P E T L E K F D K F K N L K T P D E M K A S E D L K K H G V T V L T A L G G I L K
Slow Loris G L S D G E W Q S V L N V W G K V E A D L A G H G Q E I L I R L F T A H P E T L E K F D K F K N L K T P D E M K A S E D L K K H G V T V L T A L G G I L K
Weasel Lemur G L S D G E W Q L V L N V W G K V E A D V A G H G Q E V L I R L F T G H P E T L E K F D K F K H L K T A D E M K A S E D L K K H G T T V L T A L G G I L K
Grey mouse Lemur G L S D G E W Q L V L N V W G K V E A D V A G H G Q E V L I R L F T G H P E T L E K F D K F K H L K T A D E M K A S E D L K K H G T T V L T A L G A I L K
Common Woolly Monkey G L S D G E W Q L V L N I W G K V E A D I A S H G Q E V L I S L F K G H P E T L E K F D K F K H L K S E D E M K A S E E L K K H G V T V L T A L G G I L K
Douroucouli G L S D G E W Q L V L N V W G K V E A D V A S H G Q E V L I S L F K G H P E T L E K F D K F K H L K S E D E M K A S E E L K K H G V T V L T A L G G I L K
Squirrel Monkey G L S D G E W Q L V L N I W G K V E A D I A S H G Q E V L I S L F K G H P E T L E K F D K F K H L K S E D E M K A S E E L K K H G T T V L T A L G G I L K
Brown Capuchin G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I S L F K G H P E T L E K F D K F K H L K S E D E M K A S E E L K K H G A T V L T A L G G I L K
White Tuft-Ear Marmoset G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I S L F K G H P E T L E K F D K F K H L K S E D E M K A S E E L K K H G V T V L T A L G G I L K
Rhesus monkey G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G V T V L T A L G G I L K
Crab-eating Macaque G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G V T V L T A L G G I L K
Olive Baboon G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K
Red Guenon G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K
Hanuman Langur G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K
Agile Gibbon G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K
White-cheeked gibbon G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K
Siamang G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K
Orangutan G L S D G E W Q L V L N V W G K V E A D I A S H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K
Mountain Gorilla G L S D G E W Q L V L N V W G K V E A D I A G H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K
Chimpanzee G L S D G E W Q L V L N V W G K V E A D I A G H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K
Human G L S D G E W Q L V L N V W G K V E A D I A G H G Q E V L I R L F K G H P E T L E K F D K F K H L K S E D E M K A S E D L K K H G A T V L T A L G G I L K

C (Fig. S4)


Helical position
EF
F
FG
G
GH
H
HC
Position
Echidna
Platypus
Red kangaroo
Tasmanian devil
Opossum
Aardvark
Elephant shrew
Golden mole
Cape hyrax
Florida Manatee
Steller sea cow
Dugong
African elephant
Asian Elephant
Woolly mammoth
Armadillo
Water shrew*
Eurasian shrew
Short-tailed shrew*
Hedgehog
Star-nosed mole*
Coast mole*
David's Myotis
Black flying fox
Grey Seal
Baikal Seal
Harbour Seal
Ringed seal*
Harp seal*
Hooded seal*
Bearded seal*
Weddell seal*
Northern elephant seal*
California sea lion *
Steller sea lion *
Australian sea lion*
Northern fur seal*
Walrus*
Raccoon*
Badger
American Mink*
River Otter
Black Bear
Polar Bear*
Giant Panda
Cape Fox
Bat Eared Fox
Dog
African Hunting Dog
Cat
White rhinoceros
Horse
Zebra
Pig
Cattle
Yak
Water Buffalo
Goat
Sheep
Tibetan Antelope
Red Deer
Pygmy Hippo*

D (Fig. S4)


Fin Back Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S R H P A D F G A D A Q A A M N K A L E L F R K D I A A K Y K E L G F Q G
Humpback Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S R H P A D F G A D A Q A A M N K A L E L F R K D I A A K Y K E L G F Q G
Grey Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S R H P G D F G A D A Q A A M N K A L E L F R K D I A A K Y K E L G F Q G
Pygmy Bryde's Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S R H P G D F G A D A Q A A M N K A L E L F R K D I A A K Y K E L G F Q G
Sei Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S R H P G D F G A D A Q A A M N K A L E L F R K D I A A K Y K E L G F Q G
Minke Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S R H P A E F G A D A Q A A M N K A L E L F R K D I A A K Y K E L G F Q G
Bowhead* K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S R H P G D F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G F Q G
Pygmy Sperm Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A D F G A D A Q G A M S K A L E L F R K D I A A K Y K E L G Y Q G
Dwarf Sperm Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A D F G A D A Q G A M S K A L E L F R K D I A A K Y K E L G Y Q G
Sperm whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P G D F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G Y Q G
tejneger's Beaked Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S K H P S D F G A D A Q G A M T K A L E L F R K D I A A K Y K E L G F H G
Hubbs' Beaked Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S K H P S D F G A D A Q G A M T K A L E L F R K D I A A K Y K E L G F H G
Sowerby's beaked whale* K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S K H P S D F G A D A Q G A M T K A L E L F R K D I A A K Y K E L G F H G
ongmans' Beaked Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S K H P S D F G A D A Q A A M T K A L E L F R K D I A A K Y K E L G F H G
lorthern bottlenose whale* K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S K H P S D F G A D A Q G A M T K A L E L F R K D I A A K Y K E L G F H G
Cuvier's beaked whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I I H V L H S R H P S D F G A D A Q A A M T K A L E L F R K D I A A K Y K E L G F H G
Amazon River Dolphin K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P G D F G A D A Q A A M N K A L E L F R K D I A A K Y K E L G F H G
Narwhal* K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P G D F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G F H G
Harbour Porpoise K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q G A M N K A L E L F R K D I A T K Y K E L G F H G
Dall's Porpoise K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q G A M N K A L E L F R K D I A T K Y K E L G F H G
Killer Whale K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G F H G
White beaked dolphin* K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G F H G
Risso's Dolphin* K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G F H G
Long-Fin Pilot Whale K K G H H E A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G F H G
Melon Head Whale K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G F H G
Bridled dolphin K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q A A M N K A L E L F R K D I A T K Y K E L G F H G
Saddleback Dolphin K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G F H G
Bottlenose Dolphin K K G H H D A E L K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L H S R H P A E F G A D A Q G A M N K A L E L F R K D I A A K Y K E L G F H G
Rabbit K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E A I I H V L H S K H P G D F G A D A Q A A M S K A L E L F R N D I A A Q Y K E L G F Q G
Black Lipped Pika K K G Q H E A E L K P L A Q S H A T K H K I P V K Y L E F I S E A I I Q V L Q S K H P G D F G A D A Q G A M S K A L E L F R N D M A A K Y K E L G F H G
American Pika K K G Q H E A E L K P L A Q S H A T K H K I P V K Y L E F I S E A I I Q V L Q S K H P G D F G A D A Q G A M S K A L E L F R N D M A A K Y K E L G F H G
House Mouse K K G Q H A A E I Q P L A Q S H A T K H K I P V K Y L E F I S E I I I E V L K K R H S G D F G A D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Norway Rat K K G Q H A A E I Q P L A Q S H A T K H K I P V K Y L E F I S E V I I Q V L K K R Y S G D F G A D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Chinese hamster K K G Q H A A E I Q P L A Q S H A T K H K I P V K Y L E F I S E S I I E V L K S R H S G D F G D D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Muskrat K K G H H E A E I K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L Q S K H P S N F G A D V Q G A M K R A L E L F R N D I A A K Y K E L G F Q G
Spalax carmeli K K G Q H A A E I Q P L A Q S H A T K H K I P I K Y L E F I S E A I I Q V L Q S K H P G D F G A D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Ehrenberg's Mole-Rat K K G Q H A A E I Q P L A Q S H A T K H K I P I K Y L E F I S E A I I Q V L Q S K H P G D F G A D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Kangaroo rat K K G Q H E A E I K P L A Q S H A T K H K I P M A Y L E F I S E A I I E V L K C K Y P G D F G A D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Eurasian Beaver K K G H H E A E I K P L A Q S H A T K H K I P I K Y L E F I S E A I I H V L Q S K H P G N F G A D A N G A M N K A L E L F R K D I A A K Y K E L G F Q G
Plains Viscacha K R G Q H A A E L A P L A Q S H A T K H K I P V K Y L E F I S E A I I Q V L Q S K H P G D F G A D A Q A A M S K A L E L F R N D I A A K Y K E L G F Q G
Casiragua K K G Q H A A E L A P L A Q S H A T K H K I P V K Y L E F I S E A I I Q V L Q S K H P G D F G A D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Guinea Pig K K G Q H A A E L A P L A Q S H A T K H K I P V K Y L E F I S E A I I Q V L Q S K H P G D F G A D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Northern Gundi K K G Q H E A E L A P L A Q S H A T K H K I P V K Y L E F I S E A I I Q V L E S K H P G D F G A D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Tree Shrew K K G Q H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E A I I Q V L Q S K H P G D F G A D A Q A A M S K A L E L F R N D I A A K Y K E L G F Q G
Thick Tailed Bush Baby K K G Q H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E A I I H V L Q N K H S G D F G T D V Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Potto K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E A I I H V L Q S K H P G D F G A D A Q G A M N K A L E L F R N D I A A K Y K E L G F Q G
Slow Loris K K G Q H E A E I K P L A Q S H A T K H K I P V K Y L E F I S G A I I H V L Q S K H P G D F G A D A Q G A M S K A L E L F R N D I A A K Y K E L G F Q G
Weasel Lemur K K G Q H E A E L K P L A Q S H A T K H K I P I K Y L E F I S D A I V H V L H S K H P A E F G A D A Q A A M K K A L E L F R N D I A A K Y K E L G F Q G
Grey mouse Lemur K K G Q H E A E L K P L A Q S H A T K H K I P V K Y L E F I S D A I I H V L Q S K H P A D F G A D A Q G A M K K A L E L F R N D I A A K Y K E L G F Q G
Common Woolly Monkey K K G Q H E A E L K P L A Q S H A T K H K I P V K Y L E F I S D A I I H A L Q K K H P G D F G A D A Q G A M K K A L E L F R N D M A A K Y K E L G F Q G
Douroucouli K K G H H E A E L K P L A Q S H A T K H K I P V K Y L E F I S D A I V H V L Q K K H P G D F G A D A Q G A M K K A L E L F R N D M A A K Y K E L G F Q G
Squirrel Monkey K K G Q H E A E L K P L A Q S H A T K H K I P V K Y L E L I S D A I V H V L Q K K H P G D F G A D A Q G A M K K A L E L F R N D M A A K Y K E L G F Q G
Brown Capuchin K K G Q H E A E L K P L A Q S H A T K H K I P V K Y L E F I S D A I V H V L Q K K H P G D F G A D A Q G A M K K A L E L F R N D M A A K Y K E L G F Q G
White Tuft-Ear Marmoset K K G H H E A E L K P L A Q S H A T K H K I P V K Y L E F I S D A I V H V L Q K K H P G D F G A D A Q G A M K K A L E L F R N D M A A K Y K E L G F Q G
Rhesus monkey K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E L I S E S I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R N D M A A K Y K E L G F Q G
Crab-eating Macaque K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E L I S E S I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R N D M A A K Y K E L G F Q G
Olive Baboon K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E L I S E S I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R N D M A A K Y K E L G F Q G
Red Guenon K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E L I S E S I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R N D M A A K Y K E L G F Q G
Hanuman Langur K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E L I S E S I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R N D M A A K Y K E L G F Q G
Agile Gibbon K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E C I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R K D M A S N Y K E L G F Q G
White-cheeked gibbon K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E C I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R K D M A S N Y K E L G F Q G
Siamang K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E C I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R K D M A S N Y K E L G F Q G
Orangutan K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E S I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R K D M A S N Y K E L G F Q G
Mountain Gorilla K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E C I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R K D M A S N Y K E L G F Q G
Chimpanzee K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E C I I Q V L H S K H P G D F G A D A Q G A M N K A L E L F R K D M A S N Y K E L G F Q G
Human K K G H H E A E I K P L A Q S H A T K H K I P V K Y L E F I S E C I I Q V L Q S K H P G D F G A D A Q G A M N K A L E L F R K D M A S N Y K E L G F Q G

(Fig. S4 continued)

Fig. S4

Mb amino acid alignment used for modeling Mb net surface charge and ancestral sequence reconstruction, indicating helices A to H and N-terminal (NA), inter helical (AB-GH) and C-terminal (HC) portions (top of panels *A* and *C*), with helix (above) and ordinary positional numbering (below). Internal amino acid residue positions are shaded dark (helical) or light grey (others). Major charge-increasing and charge-decreasing substitutions (boxed in blue and red, respectively; from Figs 4 and 5) that were reconstructed in diving mammals (blue background) are indicated.

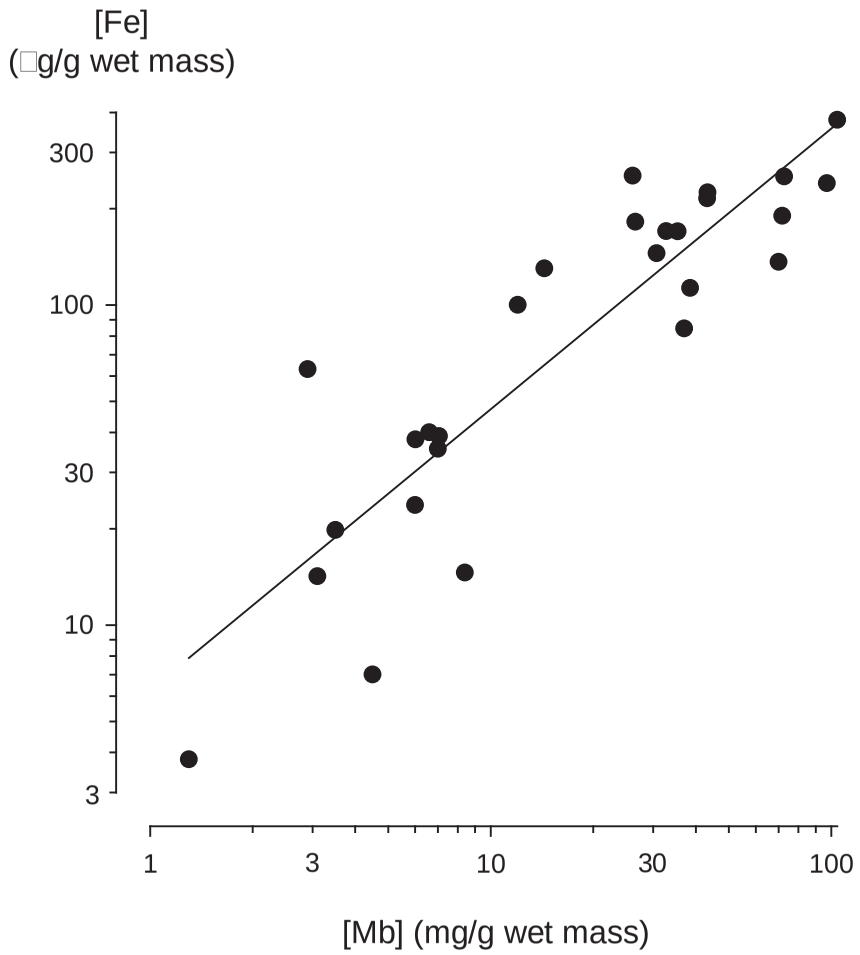


Fig. S5

Correlation between [Fe] and [Mb] in skeletal muscle of 27 mammalian diving and non-diving species. The linear regression line follows the equation log [Fe] = 0.877 log [Mb] + 0.797 (r^2 = 0.786588). See Tab. S4 for data and sources.

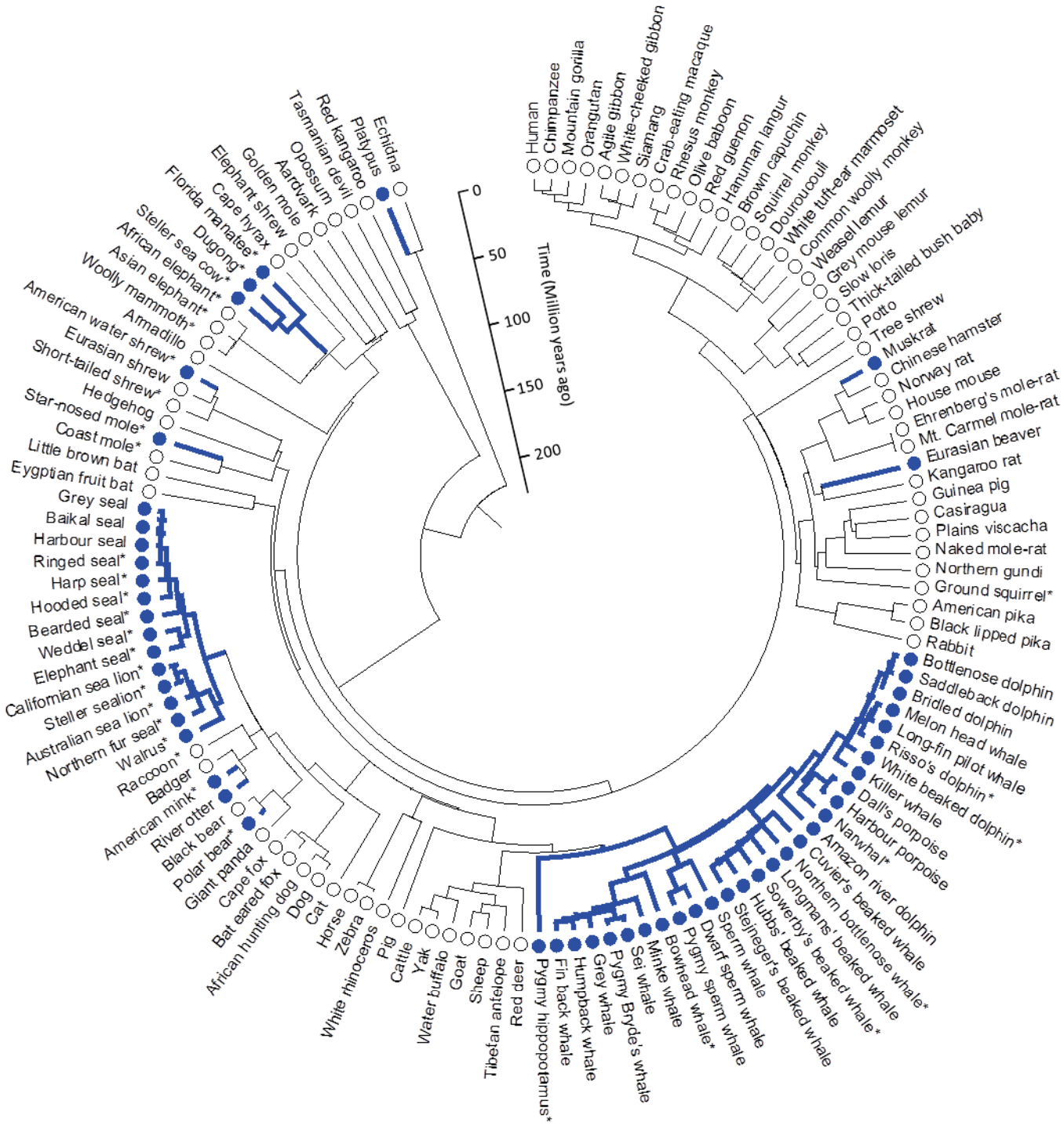


Fig. S6

Composite, time-calibrated mammalian phylogeny used for Maximum Likelihood reconstruction of ancestral Mb sequences and Mb net surface charge. Blue branches indicate 11 independent clades of aquatic or semi-aquatic mammals (filled symbols). See Table. S1 for scientific species names and Supplementary Text for tree sources. *Species whose Mb was sequenced in this study

Table S1.

Maximal dive time (t_{max}), body mass, maximal skeletal muscle Mb content ($[Mb]_{max}$), modeled Mb net surface charge (Z_{Mb}; at pH 6.5), accession number, and habitat of mammalian species considered in this study

Species	Scientific name	t_{max} (s)		Body mass (kg)		$[Mb]_{max}$ (mg/g wet mass)		Z_{Mb}	Accession No.	Habitat
Monotremata										
Echidna	*Tachyglossus aculeatus*					15.30	(*33*)	2.39	P02195	SF
Platypus	*Ornithorhynchus anatinus*	138	(*91*)	1.800	(*91*)	16.00	(*92*)	2.28	P02196	SA
Marsupialia										
Red kangaroo	*Macropus rufus*							1.53	P02194	
Tasmanian devil	*Sarcophilus harrisii*							-0.74	ENSSHAT00000006888	
Opossum	*Didelphis virginiana*							-0.74	P02193	
Placentalia										
Afrotheria										
Afroinsectiphilia										
Elephant shrew	*Elephantulus edwardii*							-0.74	AMGZ01134077	
Aquatic tenrec	*Limnogale mergulus*	15	(*93*)	0.105	(*93*)					SA
Golden mole	*Chrysochloris asiatica*							1.26	AMDV01153116	F
Aardvark	*Orycteropus afer*							0.25	ALYB01283710 (differs from previously published protein sequence P02164)	SF
Paenungulata										
Cape hyrax	*Procavia capensis*							0.39	ENSPCAT00000003677	
Amazonian manatee	*Trichechus inunguis*					9.45	(*94*)			A
Florida manatee	*Trichechus manatus latirostris*	200	(*95*)	600.000	(*96*)			-0.27	KC524769* AHIN01036638.1	A
Steller's sea cow†	*Hydrodamalis gigas*	300	(*48*)	3423.000	(*96*)			0.68	KC524738* (specimen 1) KC524739* (specimen 2) KC524740* (specimen 3)	A
Dugong	*Dugong dugon*	240	(*97*)	300.000	(*96*)			0.68	KC524735* KC524736*	A
African elephant	*Loxodonta africana*							2.11	KC524741* AAGU03077599.1	

Asian elephant	*Elephas maximus*	150	a	3050.000	(*98*)	4.60	(*99*)	2.11	(differs from previously published protein sequence P02187) KC524737*	
Woolly mammoth†	*Mammuthus primigenius*							2.11	(differs from previously published protein sequence 0605259A) KC524742* SRR006703.78243 SRR006706.169840 SRR006684.26327 SRR006668.93737 SRR006720.105927 SRR006731.115513 SRR006711.124075	
Xenarthra										
Armadillo	*Dasypus novemcinctus*					11.40	(*100*)	-0.07	AAGV03080307	SF
Laurasiatheria										
Eulipotyphla										
American water shrew	*Sorex palustris*	24	(*101*)	0.016	(*101*)	11.00	(*101*)	2.54	KC524743*	SA
Eurasian shrew	*Sorex araneus*							-0.39	AALT02095517	
Eurasian water shrew	*Neomys fodiens*	24	(*102*)	0.018	(*102*)					SA
Short-tailed shrew	*Blarina brevicauda*					8.77	(*101*)	0.14	KC524744*	
Hedgehog	*Erinaceus europaeus*							0.26	P02156	
Coast mole	*Scapanus orarius*					12.10	(*28*)	1.15	KC524746*	F
Star-nosed mole	*Condylura cristata*	59	(*28*)	0.050	(*28*)	14.40	(*28*)	2.15	KC524745*	SA/ SF
Chiroptera										
David's Myotis	*Myotis davidii*							-0.34	ELK27588	V
Black flying fox	*Pteropus alecto*							1.26	ELK10278	V
Carnivora										
Phocoidea										
Grey seal	*Halichoerus grypus*	1920	(*103*)	240.000	(*104*)	54.00	(*105*)	4.33	P68081	A
Baikal seal	*Pusa (Phoca) sibirica*	2400	(*106*)	30.000	(*106*)	67.00	(*107*)	4.33	P30562, corrected according to Baram *et al.* (*108*) and Malikov *et al.* (*109*)	A
Harbour seal	*Phoca vitulina*	1935	(*110*)	112.500	(*104*)	60.84	(*111*)	4.33	P68080	A
Ringed seal	*Pusa (Phoca) hispida*	2340	(*112*)	60.000	(*104*)	71.80	(*113*)	4.33	KC524749*	A

Harp seal	*Pagophilus groenlandicus*	1200	(*114*)	130.000	(*104*)	97.00	(*11*)	4.33	KC524750*	A
Hooded seal	*Cystophora cristata*	3120	(*115*)	300.000	(*104*)	104.00	(*11*)	4.33	KC524751*	A
Bearded seal	*Erignathus barbatus*	1122	(*116*)	225.000	(*104*)			4.20	KC524752*	A
Mediterranean monk seal	*Monachus monachus*	1080	(*117*)	325.000	(*104*)					A
Crab eater seal	*Lobodon carcinophagus*	646	(*118*)	220.000	(*104*)					A
Ross seal	*Ommatophoca rossii*	588	(*119*)	180.000	(*104*)					A
Weddell seal	*Leptonychotes weddellii*	4020	(*120*)	500.000	(*104*)	72.40	(*121*)	4.33	KC524753*	A
Northern elephant seal	*Mirounga angustirostris*	7140	(*2*)	370.000	(*122*)	79.00	(*122*)	4.20	KC524754*	A
Southern elephant seal	*Mirounga leonina*	7200	(*123*)	342.000	(*123*)					A
Otarioidea										
California sea lion	*Zalophus californianus*	594	(*124*)	110.000	(*104*)	49.00	(*125*)	4.21	P02161 KC524755*	A
Galapagos sea lion	*Zalophus wollebaeki*	360	(*126*)	91.000	(*104*)					A
Steller's sea lion	*Eumetopias jubatus*	480	(*127*)	305.000	(*104*)	49.00	(*128*)	4.21	KC524756*	A
Guadalupe fur seal	*Arctocephalus townsendi*	300	(*129*)	49.000	(*104*)					A
Juan Fernandez fur seal	*Arctocephalus philippii*	222	(*130*)	50.000	(*104*)					A
Sub-Antarctic fur seal	*Arctocephalus tropicalis*	390	(*131*)	50.000	(*104*)					A
Antarctic fur seal	*Arctocephalus gazella*	660	(*132*)	150.000	(*104*)					A
Galapagos fur seal	*Arctocephalus galapagoensis*	462	(*133*)	28.000	(*104*)					A
South American fur seal	*Arctocephalus australis*	426	(*134*)	45.000	(*104*)					A
New Zealand fur seal	*Arctocephalus forsteri*	670	(*135*)	40.000	(*104*)					A
Hooker's sea lion	*Phocarctos hookeri*	870	(*136*)	183.000	(*104*)					A
South African fur seal	*Arctocephalus pusillus*	534	(*137*)	280.000	(*104*)					A
South American sea lion	*Otaria flavescens*	462	(*138*)	119.500	(*104*)					A
Australian sea lion	*Neophoca cinerea*	540	(*139*)	61.000	(*104*)	27.00	(*140*)	4.21	KC524757*	A
Northern fur seal	*Callorhinus ursinus*	595	(*141*)	175.000	(*104*)	38.50	(*111*)	4.21	KC524758*	A
Walrus	*Odobenus rosmarus*	1440	(*142*)	1500.000	(*142*)	32.75	(*111*)	4.07	KC524759*	A
Musteloidea										
California sea otter	*Enhydra lutris*	300	(*143*)	27.700	(*144*)	31.30	(*145*)			A
Eurasian river otter	*Lutra lutra*	96	(*146*)	5.400	(*146*)	3.70	c	2.26	P11343	SA

North American river otter	*Lutra canadensis*	88	(*147*)	11.000	(*147*)					SA
Spotted-necked otter	*Lutra maculicollis*	40	(*148*)	4.000	(*148*)					SA
Marine otter	*Lontra felina*	64	(*149*)	4.500	(*149*)					SA
Cape clawless otter	*Aonyx capensis*	48	(*150*)	13.000	(*150*)					SA
American mink	*Neovison vison*	58	(*151*)	1.320	(*151*)			1.26	KC524761*	SA
Badger	*Meles meles*					2.20	c	2.56	P02157, corrected according to Dumur *et al.* (*152*)	SF
Raccoon	*Procyon lotor*					2.90	c	1.26	KC524760*	
Ursoidea										
Polar bear	*Ursus maritimus*	120	(*153*)	395.000	(*153*)	6.55	c	1.98	KC524762*	SA
Black bear	*Ursus americanus*							1.98	[TC5202]	
Giant panda	*Ailuropoda melanoleuca*							1.26	XP_002925619	
Canoidea										
Dog	*Canis lupus familiaris*					8.40	(*55*)	1.53	P63113	
African hunting dog	*Lycaon pictus*					2.00	(*154*)	1.53	P02159	
Bat eared fox	*Otocyon megalotis*							1.53	P63114	
Cape fox	*Vulpes chama*							1.53	P02160	
Feloidea										
Cat	*Felis catus*					3.97	(*155*)	0.96	GI:220426232	
Perissodactyla										
Horse	*Equus caballus*					7.05	(*156*)	1.75	P68082	
Zebra	*Equus burchellii*					7.20	(*157*)	1.75	P68083	
Tapir	*Tapirus* spp.	90	(*158*)	325.000	(*158*)					
White rhinoceros	*Ceratotherium simum*							1.67	AKZM01000633	
Cetartiodactyla										
Suina										
Pig	*Sus scrofa*					4.50	(*156*)	-0.02	NP_999401	
Ruminantia										
Greater mouse deer	*Tragulus napu*	300	(*5*)	5.000	(*98*)					
Red deer	*Cervus elaphus*							1.55	P02191, corrected to 122D (not N), as in sheep (NP_001072126) (*159*)	
Moose	*Alces alces*	45	(*160*)	600.000	(*98*)	5.32	b(*161*)			
Water buffalo	*Bubalus bubalis*					3.93	(*162*)	0.84	P84997	
Yak	*Bos grunniens*							1.83	Q2MJN4	HA

Cattle	*Bos taurus*					6.00	(*99*)	1.83	NP_776306	
Tibetan antelope	*Pantholops hodgsonii*					10.62	(*163*)	1.55	ABR24111	HA
Goat	*Capra hircus*					6.35	(*164*)	1.55	B7U9B5	
Sheep	*Ovis aries*					6.20	(*163*)	1.55	NP_001072126	
Hippopotamidae										
Hippopotamus	*Hippopotamus amphibius*	240	(*165*)	1900.000	(*98*)					SA
Pygmy hippopotamus	*Choeropsis liberiensis*							0.24	KC524763*	SA
Cetacea										
Mysticeti										
Bowhead	*Balaena mysticetus*	3660	(*166*)	79400.000	(*104*)	35.40	(*10*)	3.65	KC524747*	A
Northern minke whale	*Balaenoptera acutorostrata*	806	(*167*)	9000.000	(*98*)	7.00	(*168*)	3.94	P02179	A
Pygmy Bryde's whale	*Balaenoptera edeni*							3.65	Q0KIY2	A
Sei whale	*Balaenoptera borealis*	1200	(*169*)	23000.000	(*170*)	9.10	(*171*)	3.65	Q0KIY1	A
Grey whale	*Eschrichtius robustus*	997	(*172*)	6400.000	(*173*)			3.65	P02177	A
Humpback whale	*Megaptera novaeangliae*	1226	(*174*)	32760.000	(*104*)	16.00	(*168*)	3.65	P02178	A
Fin back whale	*Balaenoptera physalus*	1212	(*175*)	70000.000	(*98*)	37.00	(*176*)	2.94	P02180	A
Blue whale	*Balaenoptera musculus*	1617	(*177*)	70000.000	(*98*)	8.40	(*99*)			A
Odontoceti										
Physeteroidea										
Sperm whale	*Physeter catodon*	4380	(*178*)	45000.000	(*98*)	70.00	(*179*)	4.15	P02185	A
Dwarf sperm whale	*Kogia simus*	3132	(*180*)	250.000	(*104*)			4.24	P02184	A
Pygmy sperm whale	*Kogia breviceps*					43.30	(*10*)	4.24	Q0KIY5	A
Inioidea										
Amazon river dolphin	*Inia geoffrensis*							4.03	P02181	A
Lipotoidea										
Baiji	*Lipotes vexillifer*†	135	(*181*)	182.500	(*104*)					A
Delphinoidea										
Beluga	*Delphinapterus leucas*	1374	(*182*)	1150.000	(*183*)	34.40	(*10*)			A
Narwhal	*Monodon monoceros*	1572	(*184*)	1500.000	(*104*)	78.70	(*10*)	4.03	KC524748*	A
Finless porpoise	*Neophocaena phocaenoides*	149	(*185*)	35.000	(*104*)					A
Harbour porpoise	*Phocoena phocoena*	321	(*186*)	55.000	(*104*)	42.60	(*94*)	4.04	P68278	A
Dall's porpoise	*Phocoenoides dalli dalli*	167	(*187*)	50.000	(*187*)			4.04	P68277	A
Pacific white-sided dolphin	*Lagenorhynchus obliquidens*	210	(*188*)	96.000	(*188*)	34.50	(*10*)			A

Short-finned pilot whale	*Globicephala macrorhynchus*	765	(*189*)	2000.000	(*104*)					A
Irrawaddy dolphin	*Oracella brevirostris*	431	(*190*)	122.500	(*104*)					A
Killer whale	*Orcinus orca*	798	(*188*)	3850.000	(*188*)	30.70	(*10*)	4.03	P02173	A
White beaked dolphin	*Lagenorhynchus albirostris*					30.50	c	4.03	KC524767*	A
Risso's dolphin	*Grampus griseus*					26.10	c	4.03	KC524768*	A
Long-fin pilot whale	*Globicephala melas*	1678	(*191*)	1000.000	(*104*)			4.03	P02174	A
Melon head whale	*Peponocephala electra*							4.03	Q0KIY3	A
Tucuxi	*Sotalia fluviatilis*	90	(*192*)	45.000	(*98*)					A
Bridled dolphin	*Stenella attenuata*	300	(*193*)	105.000	(*104*)	25.40	(*145*)	4.03	Q0KIY7	A
Saddleback dolphin	*Delphinus delphis*					35.50	(*10*)		P68276	A
Bottlenose dolphin	*Tursiops truncatus*	408	(*188*)	189.000	(*188*)	35.34	(*194*)	4.03	P68279	A
Ziphioidea										
Cuvier's beaked whale	*Ziphius cavirostris*	5286	(*180*)	2500.000	(*104*)	43.20	(*10*)	4.80	P02182	A
Northern bottlenose whale	*Hyperoodon ampullatus*	4230	(*195*)	5800.000	(*104*)	63.40	(*176*)	4.81	KC524766*	A
Longman’s beaked whale	*Indopacetus pacificus*							4.80	Q0KIY9	A
Sowerby's beaked whale	*Mesoplodon bidens*					48.80	c	4.80	KC524765*	A
Hubbs’ beaked whale	*Mesoplodon carlhubbsi*							4.80	P02183	A
Stejneger's beaked whale	*Mesoplodon stejnegeri*							4.80	Q0KIY0	A
Blainville's beaked whale	*Mesoplodon densirostris*	3444	(*196*)	900.000	(*104*)					A
Pygmy beaked whale	*Mesoplodon peruvianus*	2228	(*180*)	1000.000	(*98*)					A
Baird's beaked whale	*Berardius bairdii*	4020	(*197*)	11000.000	(*104*)					A
Arnoux's beaked whale	*Berardius arnuxii*	4200	(*198*)	9000.000	(*104*)					A
Euarchontoglires										
Lagomorpha										
Rabbit	*Oryctolagus cuniculus*					1.30	(*199*)	0.31	P02170	SF
Plateau pika	*Ochotona curzoniae*							0.43	Q6PL31	HA/ SF
American pika	*Ochotona princeps*							0.15	P02171	HA/ SF
Rodentia										

California vole	*Microtus californicus*	20	(*200*)	0.045	(*201*)					
Muskrat	*Ondatra zibethicus*	210	(*202*)	0.681	(*202*)	14.38	(*202*)	3.60	P32428	SA
Chinese hamster	*Cricetulus griseus*							-0.19	EGW14348	SF
Australian water rat	*Hydromys chrysogaster*	32	(*203*)	0.681	(*204*)					SA
House mouse	*Mus musculus*					6.04	(*113*)	-0.19	NP_038621	
Norway rat	*Rattus norvegicus*	20	(*205*)	0.300	(*205*)	3.60	(*55*)	0.32	NP_067599	
Mt. Carmel mole-rat	*Spalax carmeli*							1.15	CAL91965	F
Ehrenberg's mole-rat	*Nannospalax ehrenbergi*					4.00	(*206*)	0.26	P04248	F
North American beaver	*Castor canadensis*	300	(*207*)	18.000	(*207*)	16.00	(*208*)			SA
Eurasian beaver	*Castor fiber*					15.61	b(*209*)	4.61	P14396	SA
Kangaroo rat	*Dipodomys ordii*							-1.67	ENSDORT00000014499	SF
Gundi	*Ctenodactylus gundi*							-1.73	P20856	
Naked mole-rat	*Heterocephalus glaber*							1.15	AFSB01158705 (exon 1) AFSB01158703 (exon 2 and 3)	F
Casiragua	*Proechimys guairae*							0.26	P04249	
Viscacha	*Lagostomus maximus*							0.26	P04250	
Guinea pig	*Cavia porcellus*					6.09	(*55*)	0.26	ENSCPOT00000006933	HA
Coypu	*Myocastor coypu*	195	(*210*)	5.000	(*211*)					SA
Capybara	*Hydrochoeris hydrochaeris*	300	(*98*)	51.000	(*98*)					SA
Ground squirrel	*Ictidomys tridecemlineatus*							1.26	ENSSTOT00000005611	SF
Scandentia										
Tree shrew	*Tupaia glis*							0.26	P02165	
Primates										
Strepsirrhini										
Thick-tailed bush baby	*Otolemur crassicaudatus*							1.28	P02168	
Potto	*Perodicticus potto edwardsi*							1.00	P02166	
Slow loris	*Nycticebus coucang*							1.28	P02167	
Weasel lemur	*Lepilemur mustelinus*							2.03	P02169	
Grey mouse lemur	*Microcebus murinus*							1.39	ENSMICT00000014099	
Haplorrhini										
Platyrrhini										
Common woolly	*Lagothrix lagotricha*							1.39	P02154	

monkey									
White tuft-ear marmoset	*Callithrix jacchus*							2.11	P02152
Douroucouli	*Aotus trivirgatus*							2.11	P02151
Squirrel monkey	*Saimiri sciureus*					2.61	(*212*)	1.39	P02155
Brown capuchin	*Cebus apella*							1.39	P02153
Catarrhini									
Cercopithecoidea									
Rhesus monkey	*Macaca mulatta*					3.37	(*213*)	0.98	XP_001081975
Crab-eating macaque	*Macaca fascicularis*	30	(*214*)	3.600	(*215*)	3.32	(*213*)	0.98	P02150
Olive baboon	*Papio anubis*							0.98	P68084
Red guenon	*Erythrocebus patas*							0.98	P68086
Hanuman langur	*Semnopithecus entellus*							0.98	P68085
Hominoidea									
Agile gibbon	*Hylobates agilis*							0.65	P62734
White-cheeked gibbon	*Nomascus leucogenys*							0.65	ENSNLET00000018342
Siamang	*Symphalangus syndactylus*							0.65	P62735
Orangutan	*Pongo pygmaeus*							0.98	P02148
Mountain gorilla	*Gorilla gorilla beringei*							0.65	P02147
Chimpanzee	*Pan troglodytes*							1.29	P02145
Human	*Homo sapiens*	150	(*216*)	49.500	(*216*)	6.45	(*217*)	0.65	NP_976311
Outgroup									
Ostrich	*Struthio camelus*							2.15	P85077
Anolis lizard	*Anolis carolinensis*							-0.33	ENSACAT00000016638

Accession numbers for GenBank, ENSEMBL and The Gene Index databases are given in plain font, underlined and in square-brackets, respectively. Conflict to previously published protein sequence is indicated in red font. Habitats are: A, aquatic; SA, semi-aquatic; HA, high-altitude; F, fossorial; SF, semi-fossorial; V, volant. Aquatic and semi-aquatic species names are in blue font. *Mb sequences obtained in this study (GenBank accession numbers KC524735-KC524769). a) Value for humans (*216*); Asian elephants during river crossings reportedly could not dive for longer than their human drivers (*218*). b) Based on Fe content using the equation log [Mb] = (log [Fe] – 0.797)/0.877, obtained from 27 diving and non-diving mammalian species with known skeletal muscle Mb and Fe contents (in mg/g and μg/g wet mass, respectively (Table S4, Fig. S5).
c) [Mb] determined in this study.

Table S2.

pK_a values of ionizable groups used for modelling Mb net surface charge (Z_{Mb}), including measurement conditions and calculated charge (*Z*)at pH 6.5.

Residue	pK_a	Mb derivative	Ionic strength (M)	*Z*
Arg	12.30 (*18*)	(average pK_a in proteins)		1.00
Lys	10.50 (*18*)	(average pK_a in proteins)		1.00
N-terminal Gly	7.72 (*219*)	MetMb Sperm whale	0.010	0.94
N terminal Ala	7.45 (*219*)	MetMb Sperm whale	0.010	0.90
N-terminal Val	7.23 (*219*)	MetMb Sperm whale	0.010	0.84
His 8	6.12 (*220*)	MetMb Minke whale, Harbour seal	0.100	0.29
His 12	6.49 (*221*)	MetMb Sperm whale	0.200	0.49
His 24	<4.50 (*222*)*	MbCO Sperm whale	0.002	<0.01
His 35	5.52 (*220*)	MetMb Dwarf sperm whale	0.100	0.09
His 36	7.89 (*221*)	MetMb Sperm whale, Horse	0.200	0.96
His 48	5.59 (*221*)	MetMb Sperm whale, Horse	0.200	0.11
His 64	<5.00 (*222*)*	MbCO Sperm whale	0.002	<0.03
His 66	6.07 a	MetMbCN Sowerby's beaked whale, Northern bottle nose whale	0.100	0.27
His 81	6.91 (*221*)	MetMb Sperm whale, Horse	0.200	0.72
His 88	7.10 a	MetMbCN Sheep	0.100	0.80
His 93	<5.00 (*222*)*	MbCO Sperm whale	0.002	<0.03
His 97	5.63 (*222*)*	MbCO Sperm whale	0.002	0.12
His 113	5.69 (*221*)	MetMb Sperm whale, Horse	0.200	0.13
His 116	6.75 (*221*)	MetMb Sperm whale, Horse	0.200	0.64
His 119	6.49 (*221*)	MetMb Sperm whale, Horse	0.200	0.49
His 128	5.53 (*220*)	MetMb California sea lion	0.100	0.10
His 152	6.10 (*220*)	MetMb Harbor seal, 6 Delphinoidei	0.100	0.28
His 34, 82, 91, 121, 124, 140	6.60 (*18*)	(average pK_a in proteins)		0.56
Tyr	10.30 (*18*)	(average pK_a in proteins)		0.00
Cys	6.80 (*18*)	(average pK_a in proteins)		-0.33
Glu	4.20 (*18*)	(average pK_a in proteins)		-1.00
Asp	3.50 (*18*)	(average pK_a in proteins)		-1.00
C-terminal	3.30 (*18*)	(average pK_a in proteins)		-1.00
Haem propionate	2.90 (*223*)	MetMb Sperm whale	0.100	-1.00

Values were obtained at 25°C, except (*) at 35°C. a) This study

Table S3.

Estimated body mass, Z_{Mb}, $[Mb]_{max}$ and t_{max} in fossil species representing transitional forms in mammalian land-to-water transitions

Species	Esti-mated body mass (kg)	Comments	Image refe-rence	Z_{Mb}	Rationale for Z_{Mb} assignment	$[Mb]_{max}$ (mg/g)	t_{max} (min)
Sirenia							
Pezosiren portelli	125	Pig-sized (*44*) (midrange for wild boar, 50 - 200 kg) (*98*)	(*44*)	2.11	similar to last common paenungulate ancestor of sirenians hyraxes and proboscideans	9	3.4
Proboscidea							
Moeritherium lyonsi	810	Tapir-sized (*35, 37*)	(*36*)	4.11	based on the gain of two additional positive charges in the probos-cidean lineage compared to the value (2.11) in the last common paen-ungulate ancestor of sirenians, hyraxes and proboscideans	26	13.7
Cetartiodactyla							
Indohyus	6.5	Raccoon-sized (*51*) (mid-range for common raccoon, usually 5 - 8 kg) (*98*)	(*51*)	1.11	similar to value at hippopotamus-whale split	6	1.1
Pakicetus attocki	43.5	Wolf-sized (*224*) (mid-range for grey wolf, 12 - 75 kg) (*98*)	(*224*)	1.11	similar to value at hippopotamus-whale split	6	1.6
Ambulocetus natans	300	Based on size of vertebrae, ribs, and limbs (*225*)	(*225*)	2.38	mid-range of values at hippopotamus-whale split and toothed whale-baleen whale split	11	4.7
Rodhocetus balochistanensis	450	Based on vertebrae (*226*)	(*227*)	2.38	mid-range of values at hippopotamus-whale split and toothed whale-baleen whale split	11	5.1
Dorudon atrox	2240	Based on vertebrae	(*228*)	3.65	similar to value at toothed whale-baleen whale split	21	13.7
Basilosaurus cetoides	6480	Based on occipital condyle breadth (*229*)	(*230*)	3.65	similar to value at toothed whale-baleen whale split	21	17.4
Pinnipedia							
Enaliarctos mealsi	80	About the size of a small male harbour seal (73 - 88 kg) (*25*)	(*25*)	2.99	similar to value at Phocoidea-Otarioidea split	15	4.7

$[Mb]_{max}$ was estimated from Z_{Mb} (Eq. 1). t_{max} was modeled on the basis of body mass and $[Mb]_{max}$ (Eq. 2).

Table S4.

Fe and Mb contents in mammalian skeletal muscle

Species	Scientific name	[Fe](μg/g wet mass)	[Mb] (mg/g wet mass)
Carnivora			
Ringed seal	*Pusa (Phoca) hispida*	190.00 (*161*)	71.80 (*113*)
Harp seal	*Pagophilus groenlandicus*	240.00 (*231*)	97.00 (*11*)
Hooded seal	*Cystophora cristata*	379.00 (*231*)	104.00 (*11*)
Weddell seal	*Leptonychotes weddellii*	252.00 (*232*)	72.70 (*121*)
Northern fur seal	*Callorhinus ursinus*	113.00 (*233*)	38.50 (*111*)
Walrus	*Odobenus rosmarus*	170.00 (*161*)	32.75 (*111*)
Raccoon	*Procyon lotor*	63.00 (*234*)	2.90 a
Polar bear	*Ursus maritimus*	40.00 (*235*)	6.60 a
Perissodactyla			
Horse	*Equus caballus*	38.90 (*236*)	7.05 (*156*)
Cetartiodactyla			
Pig	*Sus scrofa*	7.00 (*237*)	4.50 (*156*)
Sika deer	*Cervus nippon*	38.00 (*238*)	6.01 (*164*)
Moose	*Alces alces*	30.00 (*235*)	No data
Cattle	*Bos taurus*	23.70 (*237*)	6.00 (*99*)
Sheep	*Ovis aries*	19.80 (*237*)	3.50 (*163*)
Bowhead whale	*Balaena mysticetus*	169.70 (*239*)	35.40 (*10*)
Northern minke whale	*Balaenoptera acutorostrata*	35.50 (*232*)	7.00 (*168*)
Fin back whale	*Balaenoptera physalus*	84.36 (*240*)	37.00 (*176*)
Blue whale	*Balaenoptera musculus*	14.58 (*241*)	8.40 (*99*)
Sperm whale	*Physeter macrocephalus*	136.36 (*242*)	70.00 (*179*)
Pygmy sperm whale	*Kogia breviceps*	224.63 (*243*)	43.30 (*10*)
Killer whale	*Orcinus orca*	145.00 (*244*)	30.70 (*10*)
Risso’s dolphin	*Grampus griseus*	253.29 (*240*)	26.10 a
Bottlenose dolphin	*Tursiops truncatus*	181.84 (*240*)	35.34 (*194*)
Cuvier's beaked whale	*Ziphius cavirostris*	215.25 (*240*)	43.20 (*10*)
Lagomorpha			
Rabbit	*Oryctolagus cuniculus*	3.80 (*237*)	1.30 (*199*)
Rodentia			
Muskrat	*Ondatra zibethicus*	130.00 (*245*)	14.38 (*202*)
Norway rat	*Rattus norvegicus*	14.20 (*246*)	3.10 (*246*)
North American beaver	*Castor canadensis*	100.00 (*234*)	12.00 (*208*)
Eurasian beaver	*Castor fiber*	72.00 (*209*)	No data

a) This study

Table S5.

Sources of tissue samples

Species	Scientific name	Sample	Provided by
Paenungulata			
Steller's sea cow†	*Hydrodamalis gigas*	Bone-extracted ancient genomic DNA samples from three Steller's sea cow specimens housed in the Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russian Federation). ZI 6852: Sample from mandible, collected from Bering Island, Komandorskii in 1884. The best quality sample, used to obtain the complete Mb coding sequence. ZI 6853: Sample from humerus, no information on the locality Island or date. The lowest quality sample, but recovered DNA sequences match those of ZI 6852. ZI 17170(2): Sample from spine of scapula, no information on locality or date. Yielded nearly complete Mb sequences matching those of ZI 6852. Carbon 14 dating of four additional Steller's sea cow bone samples from the same collection ranged from ~680 to 1030 AD (R. MacPhee, American Museum of Natural History, unpublished data).	R. MacPhee, American Museum of Natural History
Dugong	*Dugong dugon*	Genomic DNA, female, Mabuiag Island, Torres Straits, Australia, previously extracted (*247*)	B. McDonald, James Cook University, Australia
Florida manatee	*Trichechus manatus*	Genomic DNA, female, Crystal River, Florida, USA, previously extracted (*247*)	R.K. Bonde, US Geological Survey, Gainsville, Florida
Woolly mammoth†	*Mammuthus primigenius*	Bone-extracted ancient genomic DNA previously extracted (*248*) from the mandible of a ~28,000 year old woolly mammoth recovered on the shore of Baikura-turku, Lake Taimyr, Krasnoyarsk Krai, Russian Federation [Cerpolex/Mammuthus Expeditions (CME) sample CME 2005/915, housed in the permafrost Mammoth Museum, Khatanga, Russian Federation).	H. Poinar/R. Debruyne, McMaster University
Asian elephant	*Elephas maximus*	Genomic DNA, male, previously extracted (*247*)	W. Korver, Bowmanville Zoo, Ontario
African elephant	*Loxodonta africana*	Genomic DNA, female, previously extracted (*247*)	W. Korver, Bowmanville Zoo, Ontario
Eulipotyphla			
Water shrew	*Sorex palustris*	Limb muscles, Nopiming Provincial Park, Manitoba, Canada	K. L. C.
Short-tailed shrew	*Blarina brevicauda*	Limb muscles, Whiteshell Provincial Park, Manitoba, Canada	K. L. C.
Star-nosed mole	*Condylura cristata*	Limb muscles, Nopiming Provincial Park, Manitoba, Canada	K. L. C.
Coast mole	*Scapanus orarius*	Limb muscles, Abbotsford, British Columbia,	K. L. C.

		Canada	
Phocoidea			
Grey seal	*Halichoerus grypus*	Longissimus dorsi of stranded adult male, Isle Of May, UK	D. McCafferty, University of Glasgow
Ringed seal	*Phoca hispida*	Muscle biopsy of animal of unknown age or sex, Alaska, USA	J. M. B.
Harp seal	*Pagophilus groenlandicus*	Muscle biopsy of adult female, Gulf of St. Lawrence, Canada	J. M. B.
Hooded seal	*Cystophora cristata*	Muscle biopsy of adult female, Gulf of St. Lawrence, Canada	J. M. B.
Bearded seal	*Erignathus barbatus*	Muscle biopsy of adult of unknown sex, Alaska, USA	J. M. B.
Weddell seal	*Leptonychotes weddellii*	Muscle biopsy of male pup, Big Razorback Island, Antarctica	J. M. B.
Northern elephant seal	*Mirounga angustirostris*	Muscle biopsy of pup of unknown sex, Alaska, USA	J. M. B.
Otarioidea			
California sea lion	*Zalophus californianus*	Muscle biopsy of neonate of unknown sex, Alaska, USA	J. M. B.
Steller's sea lion	*Eumetopias jubatus*	Muscle biopsy of adult of unknown sex, Alaska, USA	J. M. B.
Australian sea lion	*Neophoca cinerea*	Pectoralis, biopsy of adult female, Seal Bay Conservation Park, Kangaroo Island, South Australia	J. M. B.
Northern fur seal	*Callorhinus ursinus*	Muscle biopsy of adult female, Alaska, USA	J. M. B.
Walrus	*Odobenus rosmarus*	Muscle biopsy of adult male, Alaska, USA	J. M. B.
Musteloidea			
Eurasian river otter	*Lutra lutra*	Post mortem skeletal muscle sample of adult of unknown sex, Isle of Man, UK	K. Wareing, University of Liverpool
American mink	*Neovison vison*	Hind limb of trapped animal of unknown age and sex, Aberdeen, UK	X. Lambin, University of Aberdeen
Badger		Post mortem skeletal muscle sample of adult of unknown sex, Merseyside, Uk	K. Wareing, University of Liverpool
Raccoon	*Procyon lotor*	Forelimb of adult female obtained by hunting, Westfalen, Germany	H. Pauleickhoff
Ursoidea			
Polar bear	*Ursus maritimus*	Masseter of adult male, collected June 2008, Skagafjordur, Iceland	E. Arnason, University of Iceland
Suina			
Pig	*Sus scrofa*	Left ventricle of adult of unknown sex, Slaughterhouse, Merseyside, UK	L. Moore, University of Liverpool
Ruminatia			
Cattle	*Bos taurus*	Left ventricle of adult female, Slaughterhouse, Merseyside, UK	L. Moore, University of Liverpool
Sheep	*Ovis aries*	Left ventricle of juvenile female, Slaughterhouse, Merseyside, UK	L. Moore, University of Liverpool

Hippopotamidae			
Pygmy hippo	*Choeropsis liberiensis*	Post mortem skeletal muscle sample of juvenile of unknown sex, Whipsnade Zoo, UK	E. Flach, Zoological Society London
Mysticeti			
Bowhead whale	*Balaena mysticetus*	Biopsy from unspecified muscle of unknown sex, collected 2009, Wakeham Bay, Hudson Strait, Quebec, Canada	R. Stewart & S. Ferguson, Department of Fisheries and Oceans, Winnipeg, Canada
Northern minke whale	*Balaenoptera acutorostrata*	Muscle from caudal peduncle of stranded juvenile male, Aug. 2008, Formby, Merseyside, UK	R. Deaville, Zoological Society London
Humpback whale	*Megaptera novaeangliae*	Longissimus dorsi of stranded juvenile male, Sep. 2009, River Thames, UK	R. Deaville, Zoological Society London
Odontoceti			
Narwhal	*Monodon monoceros*	Biopsy from unspecified muscle of unknown sex, collected 2009, Arctic Bay, Nunavut, Canada	R. Stewart & S. Ferguson, Department of Fisheries and Oceans, Winnipeg, Canada
White beaked dolphin	*Lagenorhynchus albirostris*	Longissimus dorsi of stranded adult female, Cornwall, UK	R. Deaville, Zoological Society London
Risso's dolphin	*Grampus griseus*	Longissimus dorsi of stranded adult male, Sep. 2009, Anglesey, UK	R. Deaville, Zoological Society London
Northern-bottlenose whale	*Hyperoodon ampullatus*	Longissimus dorsi of stranded juvenile female, Sep. 2009, Dorset, UK	R. Deaville, Zoological Society London
Sowerby's beaked whale	*Mesoplodon bidens*	Longissimus dorsi of stranded juvenile female, September 2008, Silverdale, Morecambe Bay, UK	R. Deaville, Zoological Society London

Table S6

Primers used to amplify and sequence the protein coding region of the myoglobin gene from Cetartiodactyla, Carnivora and Eulipotyphla cDNA and from Afrotheria DNA.

Oligonucleotide	Sequence 5' ⟶ 3'
General primers	
Gen.Mb.Fwd	CTTCTTCAGACTGTGCCATG
Poly.G	GGCCACGCGTCGACTAGTACGGGGGGGG GGGG
5'.STSR3	CCGACTTCAGGTTCTTGAAC
5'.STSR1	TGGAACAGCCTGATGAGGAC
5'.CMR1	TTAAAGAGGCTGATGAGGAC
3'.Mb.Probe	CAAGTACCTGGAGTTCATCTC
SMART primer	AAGCAGTGGTATCAACGCAGAGT
Carnivora	
Car.Mb.F	CCCAGCTGTCAGAGCCAGGACACC
Car.Mb.R	CAAAGCAGACACTCAGAAGCAAAC
Pygmy hippopotamus	
Hippo.F	CAGCTGTCGGAGACAGGACACC
Hippo.R	GCTTGGATTGGGGATTTAAGG
Cetacea	
Cet.Mb.F	AGCTGTCGGAGCCAGGAYAC
Cet.Mb.R	GCCYCTCACAAACAAAGCAGG
Eulipotyphla	
Eulipotyphla.Mb.F*	GGAATTCCATATGGGGCTCAGTGATGGG GAGTGGCAGC
Eulipotyphla.Mb.R*	GTACAAGGAGCTAGGCTTCCAGGGCTAA GGATCCGGG
Woolly mammoth	
Mp.Mb.Ex1.F1	GCCAGGACACCCATTCAGTT
Mp.Mb.Ex1.R1	GATGTCAGCCTCCACTTTCCC
Mp.Mb.Ex1.F2	GAATGGGAGTTGGTGTTGAA
Mp.Mb.Ex1.R2	GAACACCCTTGAACCTTTGA
Mp.Mb.Ex2.F1	CCTGGACTTGAACTCAACCCT
Mp.Mb.Ex2.R1	TTCATCTCGCCCTCTGTCTT
Mp.Mb.Ex2.F2	AGGCTCTTCACAGGTCATCC
Mp.Mb.Ex2.R2	GGTGATGCCCTTTCTTCTTG
Mp.Mb.Ex2.F3	GTGTTACTGTGCTCACTGCCCT
Mp.Mb.Ex2.R3	CCAAATCTCATTCCCTCAGA
Mp.Mb.Ex3.F1	TCTTGTCCCCTGTGTCTGC
Mp.Mb.Ex3.R1	CATCAGCGCCAAATTCC
African & Asian elephants	
Ele.Mb.Ex1.F	GGAGAGARAATAAGAGTCGTCAGG
Ele.Mb.Ex1.R	YARTGYAGCCAACTGGTCAAC
Ele.Mb.Ex2.F	CCARCTGGGAAGCAGAGGAG
Ele.Mb.Ex2.R	GGAGGAAAGCARAGTGGRTGA
Ele.Mb.Ex3.F	GTCYRACTCYCTGCTGGCTC
Ele.Mb.Ex3.R	GCAGACACTCCAAACAAATYC

*originally developed for mouse Mb(*249*)

Table S7

Conditions used for PCR reactions

	Enzyme used	Reaction mix	Cycle conditions
cDNA amplification	Fast start High Fidelity polymerase (Roche)	5 µl 10X reaction buffer 1 µl DMSO 1 µl dNTP mix (10 mM each) 0.5 µl polymerase 2 µl each primer (10µM) 2 µl cDNA template (up to 1µg) Water up to 50 µl	Denaturation 95°C for 2 min 30 cycles: 95°C for 30 s 60°C for 30 s 72°C for 30 s final extension 72°C for 7 min
5' RACE amplification 1 (5'.STSR3)	Fast start High Fidelity polymerase (Roche)	5 µl 10X reaction buffer 1 µl DMSO 1 µl dNTP mix (10 mM each) 0.5 µl polymerase 2 µl each primer (10µM) 2 µl cDNA template (up to 1µg) Water up to 50 µl	Denaturation 95°C for 2 min 30 cycles: 95°C for 30 s 55°C for 30 s 72°C for 30 s final extension 72°C for 7 min
5' RACE amplification 1 (5'.STSR1 or 5'.CMR1)	Fast start High Fidelity polymerase (Roche)	5 µl 10X reaction buffer 1 µl DMSO 1 µl dNTP mix (10 mM each) 0.5 µl polymerase 2 µl each primer (10µM) 2 µl cDNA template (up to 1µg) Water up to 50 µl	Denaturation 95°C for 2 min 30 cycles: 95°C for 30 s 60°C for 30 s 72°C for 30 s final extension 72°C for 7 min
3' RACE	Fast start High Fidelity polymerase (Roche)	5 µl 10X reaction buffer 1 µl DMSO 1 µl dNTP mix (10 mM each) 0.5 µl polymerase 2 µl each primer (10µM) 2 µl SMART cDNA template (up to 1µg) Water up to 50 µl	Denaturation 95°C for 2 min 30 cycles: 95°C for 30 s 65°C for 30 s 72°C for 2 min final extension 72°C for 7 min
Elephant gDNA amplification	Taq DNA Polymerase (Invitrogen)	1X PCR buffer (Invitrogen) 1.5 mM $MgCl_2$ 200 µM each dNTP 0.5 µM each primer 2 U Polymerase 50 ng DNA template Water up to 20 µl	Denaturation 95°C for 3 min 30 cycles: 95°C for 30 s 55–65°C for 15 s (gradient determined) 72°C for 20 s final extension 72°C for 5 min
Woolly mammoth gDNA amplification	AmpliTaq Gold DNA Polymerase (Applied	1x GeneAmp PCR Gold Buffer 2.5 mM $MgCl_2$ 250 µM each dNTP	Denaturation 94°C for 3 min 45 cycles: 94°C for 30 s

	Biosystems)	1 µg/µl BSA 0.2 µM each primer 1 U polymerase 2 µl DNA template Water up to 50 µl	45–56°C for 30 s (gradient determined) 72°C for 40 s final extension 72°C for 7 min
Indexing PCR	Phusion High-Fidelity DNA Polymerase (Finnzymes)	1X Phusion HF Buffer 200 µM each dNTP 200 nM of primer IS4_indPCR.P5[4] 200 nM appropriate indexing primer 0.02 U/µl Polymerase 10 µl library preparation Water up to 50 µl	Denaturation 98°C for 30 s 12 cycles: 98°C for 10 s 60°C for 20 s 72°C for 20 s final extension 72°C for 10 min

References and Notes

1. S. L. Watwood, P. J. O. Miller, M. Johnson, P. T. Madsen, P. L. Tyack, Deep-diving foraging behaviour of sperm whales (*Physeter macrocephalus*). *J. Anim. Ecol.* **75**, 814 (2006). doi:10.1111/j.1365-2656.2006.01101.x Medline

2. B. S. Stewart, R. L. Delong, Double migrations of the Northern elephant seal, *Mirounga angustirostris*. *J. Mammal.* **76**, 196 (1995). doi:10.2307/1382328

3. T. M. Williams, S. R. Noren, M. Glenn, Extreme physiological adaptations as predictors of climate-change sensitivity in the narwhal, *Monodon monoceros*. *Mar. Mamm. Sci.* **27**, 334 (2011). doi:10.1111/j.1748-7692.2010.00408.x

4. P. J. Ponganis, J. U. Meir, C. L. Williams, In pursuit of Irving and Scholander: A review of oxygen store management in seals and penguins. *J. Exp. Biol.* **214**, 3325 (2011). doi:10.1242/jeb.031252 Medline

5. E. Meijaard, Umilaela, G. de Silva Wijeyeratne, Aquatic escape behaviour in mouse deer provides insight into tragulid evolution. *Mamm. Biol. Z. Saugetierkd.* **75**, 471 (2010).

6. M. D. Uhen, Evolution of marine mammals: Back to the sea after 300 million years. *Anat. Rec.* **290**, 514 (2007). doi:10.1002/ar.20545 Medline

7. P. J. Butler, D. R. Jones, Physiology of diving of birds and mammals. *Physiol. Rev.* **77**, 837 (1997). Medline

8. G. L. Kooyman, P. J. Ponganis, The physiological basis of diving to depth: Birds and mammals. *Annu. Rev. Physiol.* **60**, 19 (1998). doi:10.1146/annurev.physiol.60.1.19 Medline

9. G. L. Kooyman, E. A. Wahrenbrock, M. A. Castellini, R. W. Davis, E. E. Sinnett, Aerobic and anaerobic metabolism during voluntary diving in Weddell seals - Evidence of preferred pathways from blood-chemistry and behavior. *J. Comp. Physiol.* **138**, 335 (1980).

10. S. R. Noren, T. M. Williams, Body size and skeletal muscle myoglobin of cetaceans: Adaptations for maximizing dive duration. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **126**, 181 (2000). doi:10.1016/S1095-6433(00)00182-3 Medline

11. K. C. Lestyk, L. P. Folkow, A. S. Blix, M. O. Hammill, J. M. Burns, Development of myoglobin concentration and acid buffering capacity in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals from birth to maturity. *J. Comp. Physiol. B* **179**, 985 (2009). doi:10.1007/s00360-009-0378-9 Medline

12. J. C. Kendrew *et al*., Structure of myoglobin: A three-dimensional Fourier synthesis at 2 A. resolution. *Nature* **185**, 422 (1960). doi:10.1038/185422a0 Medline

13. G. Gros, B. A. Wittenberg, T. Jue, Myoglobin's old and new clothes: From molecular structure to function in living cells. *J. Exp. Biol.* **213**, 2713 (2010). doi:10.1242/jeb.043075 Medline

14. E. Antonini, M. Brunori, *Hemoglobin and Myoglobin in Their Reactions with Ligands*, A. Neuberger, E. L. Tatum, Eds. (Frontiers of Biology, North-Holland Publishing Company, Amsterdam, 1971).

15. E. E. Scott, E. V. Paster, J. S. Olson, The stabilities of mammalian apomyoglobins vary over a 600-fold range and can be enhanced by comparative mutagenesis. *J. Biol. Chem.* **275**, 27129 (2000). Medline

16. W. C. B. Regis, J. Fattori, M. M. Santoro, M. Jamin, C. H. I. Ramos, On the difference in stability between horse and sperm whale myoglobins. *Arch. Biochem. Biophys.* **436**, 168 (2005). doi:10.1016/j.abb.2005.01.016 Medline

17. Supplementary materials for this article are available on *Science* Online.

18. C. N. Pace, G. R. Grimsley, J. M. Scholtz, Protein ionizable groups: p*K* values and their contribution to protein stability and solubility. *J. Biol. Chem.* **284**, 13285 (2009). doi:10.1074/jbc.R800080200 Medline

19. A. H. A. Van den Oord, J. J. Wesdorp, A. F. Van Dam, J. A. Verheij, Occurrence and nature of equine and bovine myoglobin dimers. *Eur. J. Biochem.* **10**, 140 (1969). doi:10.1111/j.1432-1033.1969.tb00665.x Medline

20. S. Nagao *et al*., Structural and oxygen binding properties of dimeric horse myoglobin. *Dalton Trans.* **41**, 11378 (2012). doi:10.1039/c2dt30893b Medline

21. S. Zorrilla, G. Rivas, A. U. Acuña, M. P. Lillo, Protein self-association in crowded protein solutions: A time-resolved fluorescence polarization study. *Protein Sci.* **13**, 2960 (2004). doi:10.1110/ps.04809404 Medline

22. J. B. Wittenberg, Myoglobin-facilitated oxygen diffusion: Role of myoglobin in oxygen entry into muscle. *Physiol. Rev.* **50**, 559 (1970). Medline

23. S. R. Lavin, W. H. Karasov, A. R. Ives, K. M. Middleton, T. Garland Jr., Morphometrics of the avian small intestine compared with that of nonflying mammals: A phylogenetic approach. *Physiol. Biochem. Zool.* **81**, 526 (2008). doi:10.1086/590395 Medline

24. P. W. Hochachka, P. D. Mottishaw, in *Cold Ocean Physiology*, H.-O. Pörtner, R. C. Playle, Eds. (Cambridge Univ. Press, Cambridge, 1998), pp. 391–431.

25. A. Berta, C. E. Ray, A. R. Wyss, Skeleton of the oldest known pinniped, *Enaliarctos mealsi*. *Science* **244**, 60 (1989). doi:10.1126/science.244.4900.60 Medline

26. R. M. Kramer, V. R. Shende, N. Motl, C. N. Pace, J. M. Scholtz, Toward a molecular understanding of protein solubility: Increased negative surface charge correlates with increased solubility. *Biophys. J.* **102**, 1907 (2012). doi:10.1016/j.bpj.2012.01.060 Medline

27. P. J. Reeds, Dispensable and indispensable amino acids for humans. *J. Nutr.* **130**, 1835S (2000). Medline

28. I. W. McIntyre, K. L. Campbell, R. A. MacArthur, Body oxygen stores, aerobic dive limits and diving behaviour of the star-nosed mole (*Condylura cristata*) and comparisons with non-aquatic talpids. *J. Exp. Biol.* **205**, 45 (2002). Medline

29. M. Berenbrink, P. Koldkjaer, O. Kepp, A. R. Cossins, Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. *Science* **307**, 1752 (2005). doi:10.1126/science.1107793 Medline

30. M. Berenbrink, Evolution of vertebrate haemoglobins: Histidine side chains, specific buffer value and Bohr effect. *Respir. Physiol. Neurobiol.* **154**, 165 (2006). doi:10.1016/j.resp.2006.01.002 Medline

31. M. J. Phillips, T. H. Bennett, M. S. Lee, Molecules, morphology, and ecology indicate a recent, amphibious ancestry for echidnas. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 17089 (2009). doi:10.1073/pnas.0904649106 Medline

32. B. Campbell, The shoulder anatomy of the moles. A study in phylogeny and adaptation. *Am. J. Anat.* **64**, 1 (1939). doi:10.1002/aja.1000640102

33. P. W. Hochachka, J. Baldwin, R. I. Griffiths, Metabolic adaptations and responses of the echidna to burrowing. *Mol. Physiol.* **5**, 165 (1984).

34. A. G. Liu, E. R. Seiffert, E. L. Simons, Stable isotope evidence for an amphibious phase in early proboscidean evolution. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 5786 (2008). doi:10.1073/pnas.0800884105 Medline

35. C. W. Andrews, *A Descriptive Catalogue of the Tertiary Vertebrata of the Fayum, Egypt: Based on the Collection of the Egyptian Government in the Geological Museum, Cairo, and on the Collection in the British Museum (Natural History)* [Trustees of the British Museum (Natural History), London, 1906].

36. H. F. Osborn, *Proboscidea* (American Museum, New York, 1936), vol. 1.

37. M. T. Clementz, P. A. Holroyd, P. L. Koch, Identifying aquatic habits of herbivorous mammals through stable isotope analysis. *Palaios* **23**, 574 (2008). doi:10.2110/palo.2007.p07-054r

38. E. R. Seiffert, A new estimate of afrotherian phylogeny based on simultaneous analysis of genomic, morphological, and fossil evidence. *BMC Evol. Biol.* **7**, 224 (2007). doi:10.1186/1471-2148-7-224 Medline

39. H. F. Osborn, in *Proceedings of the Fourth International Congress of Zoology, Cambridge, 1898*, A. Sedgwick, Ed. (C. J. Clay and Sons, London, 1899), pp. 172–173.

40. M. S. Fischer, *Hyracoidea*, in *Vol. VIII: Mammalia*, in *Handbuch der Zoologie*, J. Niethammer, H. Schliemann, D. Starck, Eds. (De Gruyter, Berlin, 1992).

41. R. W. Meredith *et al*., Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification. *Science* **334**, 521 (2011); 10.1126/science.1211028. doi:10.1126/science.1211028 Medline

42. G. T. Schwartz, D. T. Rasmussen, R. J. Smith, Body-size diversity and community structure of fossil hyracoids. *J. Mammal.* **76**, 1088 (1995). doi:10.2307/1382601

43. N. N. Court, Morphology and functional anatomy of the postcranial skeleton of *Arsinoitherium*. *Palaeontogr. Abt. A Palaeozool.-Stratigr.* **226**, 125 (1993).

44. D. P. Domning, The earliest known fully quadrupedal sirenian. *Nature* **413**, 625 (2001). doi:10.1038/35098072 Medline

45. T. M. Williams, The evolution of cost efficient swimming in marine mammals: Limits to energy optimization. *Philos. Trans. R. Soc. London B Biol. Sci.* **354**, 193 (1999). doi:10.1098/rstb.1999.0371

46. P. F. Scholander, L. Irving, Experimental investigations on the respiration and diving of the Florida manatee. *J. Cell. Comp. Physiol.* **17**, 169 (1941). doi:10.1002/jcp.1030170204

47. G. J. Gallivan, J. W. Kanwisher, R. C. Best, Heart rates and gas exchange in the Amazonian manatee (*Trichechus inunguis*) in relation to diving. *J. Comp. Physiol. B* **156**, 415 (1986). Medline

48. G. W. Steller, De bestiis marinis. *Novi commentarii Academiae Scientiarvm Imperialis Petropolitanae* **2**, 289 (1751); http://gdz.sub.uni-goettingen.de/index.php?id=11&PPN=PPN350003793&DMDID=DMDLOG_0025&LOGID=LOG_0025&PHYSID=PHYS_0322&L=1.

49. L. G. Halsey, P. J. Butler, T. M. Blackburn, A phylogenetic analysis of the allometry of diving. *Am. Nat.* **167**, 276 (2006). doi:10.1086/499439 Medline

50. J. F. Schreer, K. M. Kovacs, Allometry of diving capacity in air-breathing vertebrates. *Can. J. Zool.* **75**, 339 (1997). doi:10.1139/z97-044

51. J. G. Thewissen, L. N. Cooper, M. T. Clementz, S. Bajpai, B. N. Tiwari, Whales originated from aquatic artiodactyls in the Eocene epoch of India. *Nature* **450**, 1190 (2007). doi:10.1038/nature06343 Medline

52. M. S. Lawrence, K. J. Phillips, D. R. Liu, Supercharging proteins can impart unusual resilience. *J. Am. Chem. Soc.* **129**, 10110 (2007). doi:10.1021/ja071641y Medline

53. M. Totzeck *et al*., Nitrite regulates hypoxic vasodilation via myoglobin-dependent nitric oxide generation. *Circulation* **126**, 325 (2012). doi:10.1161/CIRCULATIONAHA.111.087155 Medline

54. S. Helbo *et al*., Functional differentiation of myoglobin isoforms in hypoxia-tolerant carp indicates tissue-specific protective roles. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **302**, R693 (2012). doi:10.1152/ajpregu.00501.2011 Medline

55. B. Reynafarje, Simplified method for the determination of myoglobin. *J. Lab. Clin. Med.* **61**, 138 (1963). Medline

56. T. McLellan, Molecular charge and electrophoretic mobility in cetacean myoglobins of known sequence. *Biochem. Genet.* **22**, 181 (1984). doi:10.1007/BF00499297 Medline

57. W. Miller *et al*., Sequencing the nuclear genome of the extinct woolly mammoth. *Nature* **456**, 387 (2008). doi:10.1038/nature07446 Medline

58. N. Rohland, H. Siedel, M. Hofreiter, A rapid column-based ancient DNA extraction method for increased sample throughput. *Mol. Ecol. Resour.* **10**, 677 (2010). doi:10.1111/j.1755-0998.2009.02824.x Medline

59. M. Meyer, M. Kirchner, Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* **2010**, 10.1101/pdb.prot5448 (2010). doi:10.1101/pdb.prot5448 Medline

60. E. Hodges *et al*., Hybrid selection of discrete genomic intervals on custom-designed microarrays for massively parallel sequencing. *Nat. Protoc.* **4**, 960 (2009). doi:10.1038/nprot.2009.68 Medline

61. K. Tamura *et al*., MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731 (2011). doi:10.1093/molbev/msr121 Medline

62. W. P. Maddison, D. R. Maddison, Mesquite: A Modular System for Evolutionary Anlysis, version 2.75 (2011), http://mesquiteproject.org.

63. D. R. Maddison, W. P. Maddison, MacClade 4: Analysis of Phylogeny and Character Evolution (Sinauer Associates, Sunderland, MA, 2000).

64. M. S. Springer, W. J. Murphy, E. Eizirik, S. J. O'Brien, Placental mammal diversification and the Cretaceous-Tertiary boundary. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1056 (2003). doi:10.1073/pnas.0334222100 Medline

65. H. Nishihara, S. Maruyama, N. Okada, Retroposon analysis and recent geological data suggest near-simultaneous divergence of the three superorders of mammals. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 5235 (2009). doi:10.1073/pnas.0809297106 Medline

66. N. Poulakakis, A. Stamatakis, Recapitulating the evolution of Afrotheria: 57 genes and rare genomic changes (RGCs) consolidate their history. *Syst. Biodivers.* **8**, 395 (2010). doi:10.1080/14772000.2010.484436

67. T. Ozawa, S. Hayashi, V. M. Mikhelson, Phylogenetic position of mammoth and Steller's sea cow within Tethytheria demonstrated by mitochondrial DNA sequences. *J. Mol. Evol.* **44**, 406 (1997). doi:10.1007/PL00006160 Medline

68. N. Rohland *et al*., Proboscidean mitogenomics: Chronology and mode of elephant evolution using mastodon as outgroup. *PLoS Biol.* **5**, e207 (2007). doi:10.1371/journal.pbio.0050207 Medline

69. C. J. Douady, E. J. P. Douzery, Molecular estimation of eulipotyphlan divergence times and the evolution of "Insectivora". *Mol. Phylogenet. Evol.* **28**, 285 (2003). doi:10.1016/S1055-7903(03)00119-2 Medline

70. S. Dubey, N. Salamin, S. D. Ohdachi, P. Barrière, P. Vogel, Molecular phylogenetics of shrews (Mammalia: Soricidae) reveal timing of transcontinental colonizations. *Mol. Phylogenet. Evol.* **44**, 126 (2007). doi:10.1016/j.ympev.2006.12.002 Medline

71. M. T. Cabria, J. Rubines, B. Gómez-Moliner, R. Zardoya, On the phylogenetic position of a rare Iberian endemic mammal, the Pyrenean desman (*Galemys pyrenaicus*). *Gene* **375**, 1 (2006). doi:10.1016/j.gene.2006.01.038 Medline

72. J. H. Hutchison, Cf. *Condylura* (Mammalia: Talpidae) from the late Tertiary of Oregon. *J. Vertebr. Paleontol.* **4**, 600 (1984). doi:10.1080/02724634.1984.10012035

73. P. Colangelo *et al*., Molecular systematics and evolutionary biogeography of the genus *Talpa* (Soricomorpha: Talpidae). *Mol. Phylogenet. Evol.* **55**, 372 (2010). doi:10.1016/j.ympev.2010.01.038 Medline

74. J. W. Higdon, O. R. Bininda-Emonds, R. M. Beck, S. H. Ferguson, Phylogeny and divergence of the pinnipeds (Carnivora: Mammalia) assessed using a multigene dataset. *BMC Evol. Biol.* **7**, 216 (2007). doi:10.1186/1471-2148-7-216 Medline

75. J. J. Sato *et al*., Deciphering and dating the red panda's ancestry and early adaptive radiation of Musteloidea. *Mol. Phylogenet. Evol.* **53**, 907 (2009). doi:10.1016/j.ympev.2009.08.019 Medline

76. J. Krause *et al*., Mitochondrial genomes reveal an explosive radiation of extinct and extant bears near the Miocene-Pliocene boundary. *BMC Evol. Biol.* **8**, 220 (2008). doi:10.1186/1471-2148-8-220 Medline

77. C. Bardeleben, R. L. Moore, R. K. Wayne, A molecular phylogeny of the Canidae based on six nuclear loci. *Mol. Phylogenet. Evol.* **37**, 815 (2005). doi:10.1016/j.ympev.2005.07.019 Medline

78. U. Arnason, A. Gullberg, A. Janke, M. Kullberg, Mitogenomic analyses of caniform relationships. *Mol. Phylogenet. Evol.* **45**, 863 (2007). doi:10.1016/j.ympev.2007.06.019 Medline

79. E. Eizirik *et al*., Pattern and timing of diversification of the mammalian order Carnivora inferred from multiple nuclear gene sequences. *Mol. Phylogenet. Evol.* **56**, 49 (2010). doi:10.1016/j.ympev.2010.01.033 Medline

80. H. Zhang, L. Chen, The complete mitochondrial genome of dhole *Cuon alpinus*: Phylogenetic analysis and dating evolutionary divergence within Canidae. *Mol. Biol. Rep.* **38**, 1651 (2011). doi:10.1007/s11033-010-0276-y Medline

81. E. A. Oakenfull, J. B. Clegg, Phylogenetic relationships within the genus *Equus* and the evolution of α and θ globin genes. *J. Mol. Evol.* **47**, 772 (1998). doi:10.1007/PL00006436 Medline

82. M. Hernández Fernández, E. S. Vrba, A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. *Biol. Rev. Camb. Philos. Soc.* **80**, 269 (2005). doi:10.1017/S1464793104006670 Medline

83. M. R. McGowen, M. Spaulding, J. Gatesy, Divergence date estimation and a comprehensive molecular tree of extant cetaceans. *Mol. Phylogenet. Evol.* **53**, 891 (2009). doi:10.1016/j.ympev.2009.08.018 Medline

84. H. C. Lanier, L. E. Olson, Inferring divergence times within pikas (*Ochotona* spp.) using mtDNA and relaxed molecular dating techniques. *Mol. Phylogenet. Evol.* **53**, 1 (2009). doi:10.1016/j.ympev.2009.05.035 Medline

85. S. Horn *et al*., Mitochondrial genomes reveal slow rates of molecular evolution and the timing of speciation in beavers (*Castor*), one of the largest rodent species. *PLoS ONE* **6**, e14622 (2011). doi:10.1371/journal.pone.0014622 Medline

86. S. J. Steppan, R. M. Adkins, J. Anderson, Phylogeny and divergence-date estimates of rapid radiations in muroid rodents based on multiple nuclear genes. *Syst. Biol.* **53**, 533 (2004). doi:10.1080/10635150490468701 Medline

87. F. M. Catzeflis, E. Nevo, J. E. Ahlquist, C. G. Sibley, Relationships of the chromosomal species in the Eurasian mole rats of the *Spalax ehrenbergi* group as determined by DNA-DNA hybridization, and an estimate of the spalacid-murid divergence time. *J. Mol. Evol.* **29**, 223 (1989). doi:10.1007/BF02100206 Medline

88. H. J. Chatterjee, S. Y. Ho, I. Barnes, C. Groves, Estimating the phylogeny and divergence times of primates using a supermatrix approach. *BMC Evol. Biol.* **9**, 259 (2009). doi:10.1186/1471-2148-9-259 Medline

89. J. C. Opazo, D. E. Wildman, T. Prychitko, R. M. Johnson, M. Goodman, Phylogenetic relationships and divergence times among New World monkeys (Platyrrhini, Primates). *Mol. Phylogenet. Evol.* **40**, 274 (2006). doi:10.1016/j.ympev.2005.11.015 Medline

90. D. E. Wildman, N. M. Jameson, J. C. Opazo, S. V. Yi, A fully resolved genus level phylogeny of neotropical primates (Platyrrhini). *Mol. Phylogenet. Evol.* **53**, 694 (2009). doi:10.1016/j.ympev.2009.07.019 Medline

91. P. Bethge, S. Munks, H. Otley, S. Nicol, Diving behaviour, dive cycles and aerobic dive limit in the platypus *Ornithorhynchus anatinus*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **136**, 799 (2003). doi:10.1016/S1095-6433(03)00198-3 Medline

92. B. K. Evans, D. R. Jones, J. Baldwin, G. R. J. Gabbott, Diving ability of the platypus. *Aust. J. Zool.* **42**, 17 (1994). doi:10.1071/ZO9940017

93. J. P. Benstead, K. H. Barnes, C. M. Pringle, Diet, activity patterns, foraging movement and responses to deforestation of the aquatic tenrec *Limnogale mergulus* (Lipotyphla: Tenrecidae) in eastern Madagascar. *J. Zool.***254**, 119 (2001). doi:10.1017/S0952836901000619

94. M. H. Blessing, Studies on the concentration of myoglobin in the sea-cow and porpoise. *Comp. Biochem. Physiol. A Comp. Physiol.* **41**, 475 (1972). doi:10.1016/0300-9629(72)90005-9 Medline

95. D. S. Hartman, *Ecology and Behavior of the Manatee (Trichechus manatus) in Florida* (Special Publication No. 5, American Society of Mammologists, Pittsburgh, PA, 1979).

96. D. K. Sarko, D. P. Domning, L. Marino, R. L. Reep, Estimating body size of fossil sirenians. *Mar. Mamm. Sci.* **26**, 937 (2010). doi:10.1111/j.1748-7692.2010.00384.x

97. R. Hagihara, R. E. Jones, J. K. Sheppard, A. J. Hodgson, H. Marsh, Minimizing errors in the analysis of dive recordings from shallow-diving animals. *J. Exp. Mar. Biol. Ecol.* **399**, 173 (2011). doi:10.1016/j.jembe.2011.01.001

98. D. W. MacDonald, *The New Encyclopedia of Mammals* (Oxford Univ. Press, Oxford, 2001).

99. R. A. Lawrie, Biochemical differences between red and white muscle. *Nature* **170**, 122 (1952). doi:10.1038/170122a0 Medline

100. J. C. Seab, T. A. Burns, Myoglobin: Quantification in the nine-banded armadillo (*Dasypus novemcintus*). *Comp. Biochem. Physiol. B* **54**, 351 (1976). Medline

101. R. W. Gusztak, thesis, University of Manitoba, Winnipeg, Canada (2008).

102. R. Schloeth, Field observations of the Northern water shrew, *Neomys fodiens* (Pennant, 1771), in the Swiss National Park. *Rev. Suisse Zool.* **87**, 937 (1980).

103. D. C. Lidgard, D. J. Boness, W. D. Bowen, J. I. McMillan, M. J. I., Diving behaviour during the breeding season in the terrestrially breeding male grey seal: Implications for alternative mating tactics. *Can. J. Zool.* **81**, 1025 (2003). doi:10.1139/z03-085

104. S. H. Ridgway, R. J. Harrison, *Handbook of Marine Mammals* (Academic Press, London, 1981–1998).

105. J. Z. Reed, C. Chambers, M. A. Fedak, P. J. Butler, Gas exchange of captive freely diving grey seals (*Halichoerus grypus*). *J. Exp. Biol.* **191**, 1 (1994). Medline

106. B. S. Stewart, E. A. Petrov, E. A. Baranov, A. T. M. Ivanov, Seasonal movements and dive patterns of juvenile Baikal seals, *Phoca sibirica*. *Mar. Mamm. Sci.* **12**, 528 (1996). doi:10.1111/j.1748-7692.1996.tb00065.x

107. T. V. Neshumova, V. A. Cherepanova, E. A. Petrov, Myoglobin distribution in muscles of the seal *Pusa sibirca*. *Zh. Evol. Biokhim. Fiziol.* **19**, 93 (1983).

108. G. I. Baram, M. A. Grachev, N. G. Malikov, I. V. Nazimov, V. V. Shemyakin, Sequence of myoglobin of the seal of Lake Baikal. *Биоорган. химия* **17**, 1166 (1991).

109. N. G. Malikov, M. A. Grachev, S. M. Zelenin, I. V. Morozov, N. P. Mertvetsov, Struktura fragmentov trekh ékzonov gena mioglobina baĭkal'skoĭ nerpy *Phoca sibirica*. *Zh. Evol. Biokhim. Fiziol.* **32**, 656 (1996). Medline

110. T. Eguchi, J. T. Harvey, Diving behavior of the Pacific harbor seal (*Phoca vitulina richardsi*) in Monterey Bay, California. *Mar. Mamm. Sci.* **21**, 283 (2005). doi:10.1111/j.1748-7692.2005.tb01228.x

111. C. Lenfant, K. Johansen, J. D. Torrance, Gas transport and oxygen storage capacity in some pinnipeds and the sea otter. *Respir. Physiol.* **9**, 277 (1970). doi:10.1016/0034-5687(70)90076-9 Medline

112. J. Teilmann, E. W. Born, M. Acquarone, Behaviour of ringed seals tagged with satellite transmitters in the North Water polynya during fast-ice formation. *Can. J. Zool.* **77**, 1934 (1999).

113. P. J. O'Brien *et al*., Rapid, simple and sensitive microassay for skeletal and cardiac muscle myoglobin and hemoglobin: Use in various animals indicates functional role of myohemoproteins. *Mol. Cell. Biochem.* **112**, 45 (1992). Medline

114. L. P. Folkow, E. S. Nordøy, A. S. Blix, Distribution and diving behaviour of harp seals (*Pagophilus groenlandicus*) from the Greenland Sea stock. *Polar Biol.* **27**, 281 (2004). doi:10.1007/s00300-004-0591-7

115. L. P. Folkow, A. S. Blix, Diving behaviour of hooded seals (*Cystophora cristata*) in the Greenland and Norwegian Seas. *Polar Biol.* **22**, 61 (1999). doi:10.1007/s003000050391

116. B. A. Krafft, C. Lydersen, K. M. Kovacs, I. Gjertz, T. Haug, Diving behaviour of lactating bearded seals (*Erignathus barbatus*) in the Svalbard area. *Can. J. Zool.* **78**, 1408 (2000).

117. C. O. Kiraç, Y. Savas, H. Güçlüsoy, N. O. Veryeri, Observations on diving beahviour of free-ranging Mediterranean monk seals *Monachus monachus* on Turkish coasts. *Monachus Guardian* **5**, 37 (2005).

118. E. S. Nordøy, L. Folkow, A. S. Blix, Distribution and diving behaviour of crabeater seals (*Lobodon carcinophagus*) off Queen Maud Land. *Polar Biol.* **15**, 261 (1995). doi:10.1007/BF00239846

119. J. L. Bengtson, B. S. Stewart, Diving patterns of a Ross seal (*Ommatophoca rossii*) near the eastern coast of the Antarctic Peninsula. *Polar Biol.* **18**, 214 (1997). doi:10.1007/s003000050178

120. J. Plötz, H. Bornemann, R. Knust, A. Schröder, M. Bester, Foraging behaviour of Weddell seals, and its ecological implications. *Polar Biol.* **24**, 901 (2001). doi:10.1007/s003000100297

121. S. B. Kanatous *et al*., The ontogeny of aerobic and diving capacity in the skeletal muscles of Weddell seals. *J. Exp. Biol.* **211**, 2559 (2008). doi:10.1242/jeb.018119 Medline

122. J. L. Hassrick *et al*., Condition and mass impact oxygen stores and dive duration in adult female northern elephant seals. *J. Exp. Biol.* **213**, 585 (2010). doi:10.1242/jeb.037168 Medline

123. D. J. Slip, M. A. Hindell, H. R. Burton, in *Elephant Seals: Population Ecology, Behavior, and Physiology*, B. J. Le Boeuf, R. Laws, Eds. (Univ. of California Press, Los Angeles, 1994), pp. 253–270.

124. S. D. Feldkamp, R. L. De Long, G. A. Antonelis, Diving patterns of California sea lions, *Zalophus californianus*. *Can. J. Zool.* **67**, 872 (1989). doi:10.1139/z89-129

125. M. J. Weise, D. P. Costa, Total body oxygen stores and physiological diving capacity of California sea lions as a function of sex and age. *J. Exp. Biol.* **210**, 278 (2007). doi:10.1242/jeb.02643 Medline

126. G. L. Kooyman, F. Trillmich, in *Fur Seals: Maternal Strategies on Land and at Sea*, R. L. Gentry, G. L. Kooyman, Eds. (Princeton University Press, Princeton, NJ, 1986), pp. 209–218.

127. R. L. Merrick, T. R. Loughlin, Foraging behavior of adult female and young-of-the-year Steller sea lions in Alaskan waters. *Can. J. Zool.* **75**, 776 (1997). doi:10.1139/z97-099

128. J. P. Richmond, J. M. Burns, L. D. Rea, Ontogeny of total body oxygen stores and aerobic dive potential in Steller sea lions (*Eumetopias jubatus*). *J. Comp. Physiol. B* **176**, 535 (2006). doi:10.1007/s00360-006-0076-9 Medline

129. M. E. Lander, F. M. Gulland, R. L. De Long, Satellite tracking a rehabilitated Guadalupe fur seal (*Arctocephalus townsendi*). *Aquat. Mamm.* **26**, 137 (2000).

130. J. Francis, D. Boness, H. Ochoa-Acuna, A protracted foraging and attendance cycle in female Juan Fernandez fur seals. *Mar. Mamm. Sci.* **14**, 552 (1998). doi:10.1111/j.1748-7692.1998.tb00742.x

131. J.-Y. Georges, Y. Tremblay, C. Guinet, Seasonal diving behaviour in lactating subantarctic fur seals on Amsterdam Island. *Polar Biol.* **23**, 59 (2000). doi:10.1007/s003000050008

132. I. L. Boyd, J. P. Croxall, Dive durations in pinnipeds and seabirds. *Can. J. Zool.* **74**, 1696 (1996). doi:10.1139/z96-187

133. G. L. Kooyman, F. Trillmich, in *Fur Seals: Maternal Strategies on Land and at Sea*, R. L. Gentry, G. L. Kooyman, Eds. (Princeton Univ. Press, Princeton, NJ, 1986), pp. 186–195.

134. F. Trillmich, G. L. Kooyman, P. Majluf, M. Sanchez-Griñan, in *Fur Seals: Maternal Strategies on Land and at Sea*, R. L. Gentry, G. L. Kooyman, Eds. (Princeton Univ. Press, Princeton, NJ, 1986), pp. 154–167.

135. R. Mattlin, N. J. Gales, D. P. Costa, Seasonal dive behaviour of lactating New Zealand fur seals (*Arctocephalus forsteri*). *Can. J. Zool.* **76**, 350 (1998).

136. B. L. Chilvers, I. S. Wilkinson, P. J. Duignan, N. J. Gemmell, Diving to extremes: Are New Zealand sea lions (*Phocarctos hookeri*) pushing their limits in a marginal environment. *J. Zool.* **269**, 233 (2006).

137. J. P. Y. Arnould, M. A. Hindell, Dive behaviour, foraging locations, and maternal-attendance patterns of Australian fur seals (*Arctocephalus pusillus doriferus*). *Can. J. Zool.* **79**, 35 (2001).

138. R. Werner, C. Campagna, Diving behaviour of lactating southern sea lions (*Otaria flavescens*) in Patagonia. *Can. J. Zool.* **73**, 1975 (1995). doi:10.1139/z95-232

139. S. L. Fowler, D. P. Costa, J. P. Arnould, N. J. Gales, C. E. Kuhn, Ontogeny of diving behaviour in the Australian sea lion: Trials of adolescence in a late bloomer. *J. Anim. Ecol.* **75**, 358 (2006). doi:10.1111/j.1365-2656.2006.01055.x Medline

140. S. L. Fowler, D. P. Costa, J. P. Y. Arnould, N. J. Gales, J. M. Burns, Ontogeny of oxygen stores and physiological diving capability in Australian sea lions. *Funct. Ecol.* **21**, 922 (2007). doi:10.1111/j.1365-2435.2007.01295.x

141. J. Sterling, R. Ream, At-sea behavior of juvenile male northern fur seals (*Callorhinus ursinus*). *Can. J. Zool.* **82**, 1621 (2004). doi:10.1139/z04-136

142. I. Gjertz, D. Griffiths, B. A. Krafft, C. Lydersen, Ø. Wiig, Diving and haul-out patterns of walruses *Odobenus rosmarus* on Svalbard. *Polar Biol.* **24**, 314 (2001). doi:10.1007/s003000000211

143. K. L. Laidre, R. J. Jameson, Foraging patterns and prey selection in an increasing and expanding sea otter population. *J. Mammal.* **87**, 799 (2006). doi:10.1644/05-MAMM-A-244R2.1

144. L. C. Yeates, T. M. Williams, T. L. Fink, Diving and foraging energetics of the smallest marine mammal, the sea otter (*Enhydra lutris*). *J. Exp. Biol.* **210**, 1960 (2007). doi:10.1242/jeb.02767 Medline

145. M. A. Castellini, G. N. Somero, Buffering capacity of vertebrate muscle: Correlations with potentials for anaerobic function. *J. Comp. Physiol.* **143**, 191 (1981).

146. B. A. Nolet, D. E. H. Wansink, H. Kruuk, Diving of otters (*Lutra lutra*) in a marine habitat: Use of depths by a single-prey loader. *J. Anim. Ecol.* **62**, 22 (1993). doi:10.2307/5479

147. M. Ben-David, T. M. Williams, O. A. Ormseth, Effects of oiling on exercise physiology and diving behaviour of river otters: a captive study. *Can. J. Zool.* **78**, 1380 (2000). doi:10.1139/z00-059

148. D. T. Rowe-Rowe, M. J. Somers, in *Behaviour and Ecology of Riparian Mammals*, N. Dunstone, M. Gorman, Eds. (Cambridge Univ. Press, Cambridge, 1998), pp. 215–228.

149. H. Kruuk, *Otters: Ecology, Behaviour and Conservation* (Oxford Univ. Press, Oxford, 2006).

150. M. J. Somers, Foraging behaviour of Cape clawless otters (*Aonyx capensis*) in a marine habitat. *J. Zool. (Lond.)* **252**, 473 (2000). doi:10.1111/j.1469-7998.2000.tb01230.x

151. L. A. Harrington *et al*., Dive performance in a small-bodied, semi-aquatic mammal in the wild. *J. Mammal.* **93**, 198 (2012). doi:10.1644/10-MAMM-A-351.1

152. V. Dumur, M. Dautrevaux, K. Han, The covalent structure of dog myoglobin. *Biochim. Biophys. Acta* **420**, 376 (1976). doi:10.1016/0005-2795(76)90329-9 Medline

153. G. B. Kolenosky, in *Wild Furbearer Managment and Conservation in North America*, M. Novak, J. A. Baker, M. E. Obbard, B. Malloch, Eds. (Ministry of Natural Resources, Ontario, Canada, 1987), pp. 474–485.

154. T. M. Williams *et al*., Skeletal muscle histology and biochemistry of an elite sprinter, the African cheetah. *J. Comp. Physiol. B* **167**, 527 (1997). doi:10.1007/s003600050105 Medline

155. D. J. Reis, G. F. Wooten, The relationship of blood flow to myoglobin, capillary density, and twitch characteristics in red and white skeletal muscle in cat. *J. Physiol.* **210**, 121 (1970). Medline

156. R. A. Lawrie, Some observations on factors affecting myoglobin concentrations in muscle. *J. Agric. Sci.* **40**, 356 (1950). doi:10.1017/S0021859600046116

157. C. A. Onyango, M. Izumimoto, P. M. Kutima, Comparison of some physical and chemical properties of selected game meats. *Meat Sci.* **49**, 117 (1998). doi:10.1016/S0309-1740(97)00116-2 Medline

158. R. A. Barongi, Husbandry and conservation of tapirs. *Int. Zoo Yearb.* **32**, 7 (1993).

159. W. Vötsch, F. A. Anderer, The amino acid sequence of myoglobin from skeletal muscles of red deer (*Cervus elaphus*). *J. Mol. Evol.* **5**, 315 (1975). doi:10.1007/BF01732218 Medline

160. A. de Vos, Summer observations on moose behavior in Ontario. *J. Mammal.* **39**, 128 (1958). doi:10.2307/1376618

161. H. V. Kuhnlein, H. M. Chan, D. Leggee, V. Barthet, Macronutrient, mineral and fatty acid composition of Canadian Arctic traditional food. *J. Food Compost. Anal.* **15**, 545 (2002).

162. R. Dosi *et al*., Characterization and kinetics studies of water buffalo (*Bubalus bubalis*) myoglobin. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **145**, 230 (2006). doi:10.1016/j.cbpb.2006.07.006 Medline

163. L. Ma, Y.-Z. Yang, G.-E. Jin, R.-L. Ge, Comparison on the myoglobin contents and lactate dehydrogenase activity in cardiac and skeletal muscles between Tibetan antelope and Tibetan sheep. *Chin. J. Zool.* **47**, 35 (2012).

164. K. Shimada *et al*., Species and muscle differences in L-carnitine levels in skeletal muscles based on a new simple assay. *Meat Sci.* **68**, 357 (2004). doi:10.1016/j.meatsci.2004.04.003 Medline

165. A. E. Brehm, *Brehms Thierleben, 1. Abtheilung Säugethiere* (Verlag des Bibliographischen Instituts, Leipzig, Germany, ed. 2, 1880), vol. 3.

166. G. K. Krutzikowsky, B. R. Mate, Dive and surfacing characteristics of bowhead whales (*Balaena mysticetus*) in the Beaufort and Chukchi seas. *Can. J. Zool.* **78**, 1182 (2000).

167. K. Stockin, R. Fairbairns, E. Parsons, D. Sims, Effects of diel and seasonal cycles on the dive duration of the minke whale (*Balaenoptera acutorostrata*). *J. Mar. Biol. Assoc. U. K.* **81**, 189 (2001). doi:10.1017/S0025315401003630

168. S. Helbo, A. Fago, Functional properties of myoglobins from five whale species with different diving capacities. *J. Exp. Biol.* **215**, 3403 (2012). doi:10.1242/jeb.073726 Medline

169. A. R. Martin, *An Illustrated Encyclopedia of Whales and Dolphins* (Portland House, New York, 1990).

170. C. Lockyer, T. Waters, Weights and anatomical measurements of Northeastern Atlantic fin (*Balaenoptera physalus*, Linnaeus) and sei (*B. borealis*, Lesson) whales. *Mar. Mamm. Sci.* **2**, 169 (1986). doi:10.1111/j.1748-7692.1986.tb00039.x

171. T. Tawara, On the respiratory pigments of whales (Studies on whale blood II.). *Sci. Rep. Whales Res. Inst.* **3**, 96 (1950).

172. W. E. Evans, Telemetering of temperature and depth data from a free ranging yearling California gray whale, *Eschrichtius robustus*. *Mar. Fish. Rev.* **36**, 52 (1974).

173. J. L. Sumich, T. Goff, W. L. Perryman, Growth of two captive gray whale calves. *Aquat. Mamm.* **2001**, 231 (2001).

174. W. F. Dolphin, Foraging dive patterns of humpback whales, *Megaptera novaeangliae*, in southeast Alaska: a cost - benefit analysis. *Can. J. Zool.* **66**, 2432 (1988). doi:10.1139/z88-360

175. A. H. Kopelman, S. Sadove, Ventilatory rate differences between surface-feeding and non-surface-feeding fin whales (*Balaenoptera physalus*) in the waters off eastern Long Island, New York, U.S.A. *Mar. Mamm. Sci.* **11**, 200 (1995). doi:10.1111/j.1748-7692.1995.tb00518.x

176. P. F. Scholander, Experimental investigations on the respiratory function in diving mammals and birds. *Hvalrådets Skr.* **22**, 1 (1940).

177. G. Donovan, Blue whales off Peru, December 1982, with special reference to pygmy blue whales. *Rep. Int. Whaling Comm.* **34**, 473 (1984).

178. W. A. Watkins, M. A. Daher, K. M. Fristrup, T. J. Howald, G. N. Disciara, Sperm whales tagged with transponders and tracked underwater by sonar. *Mar. Mamm. Sci.* **9**, 55 (1993). doi:10.1111/j.1748-7692.1993.tb00426.x

179. J. G. Sharp, B. B. Marsh, *Whalemeat: Production and Preservation* (UK Department of Scientific and Industrial Research, London, UK, 1953).

180. J. Barlow, K. Forney, A. V. Saunder, J. Urban-Ramirez, "A report of cetacean acoustic detection and dive interval studies (CADDIS) conducted in the Southern Gulf of

California, 1995" (NOAA-TM-NMFS-SWFC-250, U.S. Department of Commerce, La Jolla, CA, 1997).

181. C. Peixun, in *Handbook of Marine Mammals*, S. H. Ridgway, R. J. Harrison, Eds. (Academic Press, London, 1989), pp. 25–44.

182. A. R. Martin, T. Smith, Strategy and capability of wild belugas, *Delphinapterus leucas*, during deep, benthic diving. *Can. J. Zool.* **77**, 1783 (1999).

183. R. Reeves, B. Stewart, P. Clapham, J. Powell, *Guide to Marine Mammals of the World* (Knopf, New York, 2003).

184. K. L. Laidre, M. Heide-Jorgensen, R. Dietz, Diving behaviour of narwhals (*Monodon monoceros*) at two coastal localities in the Canadian High Arctic. *Can. J. Zool.* **80**, 624 (2002). doi:10.1139/z02-041

185. T. Akamatsu, D. Wang, K. Wang, Q. Zhao, Y. Naito, Diving behaviour of freshwater finless porpoises (*Neophocaena phocaenoides*) in an oxbow of the Yangtze River, China. *ICES J. Mar. Sci.* **59**, 438 (2002). doi:10.1006/jmsc.2001.1159

186. A. J. Westgate, A. J. Read, P. Berggren, H. N. Koopman, D. E. Gaskin, Diving behaviour of harbour porpoises, *Phocoena phocoena*. *Can. J. Fish. Aquat. Sci.* **52**, 1064 (1995). doi:10.1139/f95-104

187. M. B. Hanson, R. W. Baird, Dall's porpoise reactions to tagging attempts using a remotely-deployed suction-cup tag. *Mar. Technol. Soc. J.* **32**, 18 (1998).

188. S. R. Noren *et al*., Changes in partial pressures of respiratory gases during submerged voluntary breath hold across odontocetes: is body mass important? *J. Comp. Physiol. B* **182**, 299 (2012). doi:10.1007/s00360-011-0612-0 Medline

189. C. A. Bowers, R. S. Henderson, "Project Deep OPS: Deep object recovery with pilot and killer whales" (NUC TP 306, Naval Undersea Center, Hawaii Laboratory, Kaneohe, HI, 1972).

190. P. J. Stacey, G. T. Hvenegaard, Habitat use and behaviour of Irrawady dolphins (*Orcaella brevirostris*) in the Mekong river of Laos. *Aquat. Mamm.* **28**, 1 (2002).

191. B. R. Mate, B. A. Lagerquist, M. Winsor, J. Geraci, J. H. Prescott, Notes: Movement and dive habits of a satellite-monitored longfinned pilot whale (*Globicephala melas*) in the northwest Atlantic. *Mar. Mamm. Sci.* **21**, 136 (2005). doi:10.1111/j.1748-7692.2005.tb01213.x

192. H. H. Edwards, G. D. Schnell, Body length, swimming speed, dive duration, and coloration of the dolphin *Sotalia fluviatilis* (Tucuxi) in Nicaragua. *Caribb. J. Sci.* **37**, 271 (2001).

193. R. W. Baird, A. D. Ligon, S. K. Hooker, A. M. Gorgone, Subsurface and nighttime behaviour of pantropical spotted dolphins in Hawaii. *Can. J. Zool.* **79**, 988 (2001). doi:10.1139/z01-070

194. L. Eichelberger, E. S. Fetcher, E. M. K. Geiling, B. J. Vos, Muscle and blood hemoglobin in the dolphin. *Science* **90**, 443 (1939). doi:10.1126/science.90.2341.443 Medline

195. S. K. Hooker, R. W. Baird, Deep-diving behaviour of the northern bottlenose whale, *Hyperoodon ampullatus* (Cetacea: Ziphiidae). *Proc. R. Soc. Lond. B Biol. Sci.* **266**, 671 (1999). doi:10.1098/rspb.1999.0688

196. P. L. Tyack, M. Johnson, N. A. Soto, A. Sturlese, P. T. Madsen, Extreme diving of beaked whales. *J. Exp. Biol.* **209**, 4238 (2006). doi:10.1242/jeb.02505 Medline

197. T. Kasuya, Distribution and behavior of Baird's beaked whales off the Pacific coast of Japan. *Rep. Int. Whaling Comm.* **37**, 61 (1986).

198. R. P. Hobson, A. R. Martin, Behaviour and dive times of Arnoux's beaked whales, *Berardius arnuxii*, at narrow leads in fast ice. *Can. J. Zool.* **74**, 388 (1996). doi:10.1139/z96-045

199. G. T. Perkoff, F. H. Tyler, Estimation and physical properties of myoglobin in various species. *Metabolism* **7**, 751 (1958). Medline

200. G. F. Fisler, Behavior of salt-marsh *Microtus* during winter high tides. *J. Mammal.* **42**, 37 (1961). doi:10.2307/1377238

201. B. J. Verts, L. N. Carraway, *Land Mammals of Oregon* (Univ. California Press, Berkeley, CA, 1998).

202. R. A. MacArthur, M. M. Humphries, G. A. Fines, K. L. Campbell, Body oxygen stores, aerobic dive limits, and the diving abilities of juvenile and adult muskrats (*Ondatra zibethicus*). *Physiol. Biochem. Zool.* **74**, 178 (2001). doi:10.1086/319662 Medline

203. D. Petzold, thesis, University of Bielefeld, Bielefeld, Germany (1995).

204. C. A. Atkinson, M. A. Lund, K. D. Morris, BiblioRakali: The Australian water rat, *Hydromys chrysogaster* Geoffroy, 1804 (Muridae: Hydromyinae), a subject-specific bibliography. *Conserv. Sci. West. Australia* **7**, 65 (2008).

205. W. M. Panneton *et al*., Activation of brainstem neurons by underwater diving in the rat. *Front. Physiol.* **3**, 111 (2012). doi:10.3389/fphys.2012.00111 Medline

206. A. Ar, R. Arieli, A. Shkolnik, Blood-gas properties and function in the fossorial mole rat under normal and hypoxic-hypercapnic atmospheric conditions. *Respir. Physiol.* **30**, 201 (1977). doi:10.1016/0034-5687(77)90031-7 Medline

207. L. Irving, M. D. Orr, The diving habits of the beaver. *Science* **82**, 569 (1935). doi:10.1126/science.82.2137.569 Medline

208. T. McKean, C. Carlton, Oxygen storage in beavers. *J. Appl. Physiol.* **42**, 545 (1977). Medline

209. W. Korzeniowski, B. Jankowska, A. Kwiatkowska, T. Zmijewski, Chemical composition of European beaver (*Castor fiber* L.) meat. *Nat. Sci.* **3**, 291 (1999).

210. C. A. Sooter, Nutria good diver. *J. Mammal.* **24**, 503 (1943).

211. F. L. Ferrante, Oxygen conservation during submergence apnea in a diving mammal, the nutria. *Am. J. Physiol.* **218**, 363 (1970). Medline

212. R. R. J. Chaffee, Y. Cassuto, S. M. Horvath, Studies on the effects of cold acclimation on myoglobin levels in sparrows, mice, hamsters, and monkeys. *Can. J. Physiol. Pharmacol.* **43**, 1021 (1965). doi:10.1139/y65-108

213. L. J. Kagen, R. Gurevich, Precipitin reactions of anti-human myoglobin serum with several human and animal muscle extracts. *Immunology* **12**, 667 (1967). Medline

214. A. Fothergill, in *Planet Earth* (BBC Natural History Unit, Bristol, 2006), vol. 3.

215. J. E. Fa, The genus *Macaca*: A review of taxonomy and evolution. *Mammal Rev.* **19**, 45 (1989). doi:10.1111/j.1365-2907.1989.tb00401.x

216. G. Teruoka, Die Ama und ihre Arbeit. *Arbeitsphysiologie* **5**, 239 (1932).

217. M. H. Blessing, Bindegewebsgehalt und Myoglobinkonzentration menschlicher Skeletmuskulatur. *Virchows Archiv. A Pathol. Anat.* **352**, 130 (1971). Medline

218. J. H. Williams, *Elephant Bill* (Doubleday, New York, 1950).

219. M. R. Busch, D. G. Maskalick, G. W. Neireiter, D. E. Harris, F. R. N. Gurd, Aliphatic semisynthetic variants of the amino-terminal residue of sperm whale myoglobin: Enrichment with carbon-13 and determination and interpretation of terminal pK values. *Biochemistry* **24**, 6707 (1985). doi:10.1021/bi00344a061 Medline

220. L. H. Botelho *et al*., Proton nuclear magnetic resonance study of histidine ionizations in myoglobins of various species. Comparison of observed and computed p*K* values. *Biochemistry* **17**, 5197 (1978). doi:10.1021/bi00617a020 Medline

221. Y. H. Kao *et al*., Salt effects on ionization equilibria of histidines in myoglobin. *Biophys. J.* **79**, 1637 (2000). doi:10.1016/S0006-3495(00)76414-9 Medline

222. D. Bashford, D. A. Case, C. Dalvit, L. Tennant, P. E. Wright, Electrostatic calculations of side-chain pK_a values in myoglobin and comparison with NMR data for histidines. *Biochemistry* **32**, 8045 (1993). doi:10.1021/bi00082a027 Medline

223. S. H. Friend, F. R. N. Gurd, Electrostatic stabilization in myoglobin. pH dependence of summed electrostatic contributions. *Biochemistry* **18**, 4612 (1979). doi:10.1021/bi00588a023 Medline

224. J. G. M. Thewissen, E. M. Williams, L. J. Roe, S. T. Hussain, Skeletons of terrestrial cetaceans and the relationship of whales to artiodactyls. *Nature* **413**, 277 (2001). doi:10.1038/35095005 Medline

225. J. G. M. Thewissen, S. T. Hussain, M. Arif, Fossil evidence for the origin of aquatic locomotion in archaeocete whales. *Science* **263**, 210 (1994). doi:10.1126/science.263.5144.210 Medline

226. P. D. Gingerich, Land-to-sea transition in early whales: Evolution of Eocene Archaeoceti (Cetacea) in relation to skeletal proportions and locomotion of living semiaquatic mammals. *Paleobiology* **29**, 429 (2003). doi:10.1666/0094-8373(2003)029<0429:LTIEWE>2.0.CO;2

227. P. D. Gingerich, I. S. Haq Mu, I. H. Zalmout, M. S. Khan, Malkani, Origin of whales from early artiodactyls: Hands and feet of Eocene Protocetidae from Pakistan. *Science* **293**, 2239 (2001). doi:10.1126/science.1063902 Medline

228. P. D. Gingerich *et al*., New protocetid whale from the middle eocene of pakistan: Birth on land, precocial development, and sexual dimorphism. *PLoS ONE* **4**, e4366 (2009). doi:10.1371/journal.pone.0004366 Medline

229. L. Marino, D. W. McShea, M. D. Uhen, Origin and evolution of large brains in toothed whales. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* **281**, 1247 (2004). doi:10.1002/ar.a.20128 Medline

230. R. Kellogg, *A Review of the Archaeoceti* (Carnegie Institution of Washington, Washington, DC, 1936).

231. L. A. Brunborg, K. Julshamn, R. Nortvedt, L. Frøyland, Nutritional composition of blubber and meat of hooded seal (*Cystophora cristata*) and harp seal (*Phagophilus groenlandicus*) from Greenland. *Food Chem.* **96**, 524 (2006). doi:10.1016/j.foodchem.2005.03.005

232. K. Honda, Y. Yamamoto, R. Tatsukawa, Distribution of heavy metals in Antarctic marine ecosystem. *Proc. Nat. Inst. Polar Res. Symp. Polar Biol.* **1**, 184 (1987).

233. K. Noda *et al*., Distribution of heavy metals in muscle, liver and kidney of northern fur seal (*Callorhinus ursinus*) caught off Sanriku, Japan and from the Pribilof Islands, Alaska. *Environ. Pollut.* **90**, 51 (1995). doi:10.1016/0269-7491(94)00089-V Medline

234. C. D. Wren, H. R. MacCrimmon, B. R. Loescher, Examination of bioaccumulation and biomagnification of metals in a Precambrian Shield lake. *Water Air Soil Pollut.* **19**, 277 (1983). doi:10.1007/BF00599055

235. H. V. Kuhnlein, R. Soueida, Use and nutrient composition of traditional Baffin Inuit foods. *J. Food Compost. Anal.* **5**, 112 (1992). doi:10.1016/0889-1575(92)90026-G

236. A. Badiani, N. Nanni, P. P. Gatta, B. Tolomelli, M. Manfredini, Nutrient profile of horsemeat. *J. Food Compost. Anal.* **10**, 254 (1997). doi:10.1006/jfca.1997.0540

237. G. Lombardi-Boccia, S. Lanzi, A. Aguzzi, Aspects of meat quality: Trace elements and B vitamins in raw and cooked meats. *J. Food Compost. Anal.* **18**, 39 (2005). doi:10.1016/j.jfca.2003.10.007

238. Y. Ishizuka, Y. Kawai, S. Ohtani, M. Irie, Composition, mineral contents and color of muscles of wild Sika deer (*Cervus nippon centralis*). *Anim. Sci. J.* **72**, J551 (2001).

239. T. M. O'Hara, C. Hanns, G. Bratton, R. Taylor, V. M. Woshner, Essential and non-essential elements in eight tissue types from subsistence-hunted bowhead whale: nutritional and toxicological assessment. *Int. J. Circumpolar Health* **65**, 228 (2006). Medline

240. R. Capelli *et al*., Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios. *Sci. Total Environ.* **390**, 569 (2008). doi:10.1016/j.scitotenv.2007.10.036 Medline

241. S. Nicol *et al*., Southern Ocean iron fertilization by baleen whales and Antarctic krill. *Fish Fish.* **11**, 203 (2010). doi:10.1111/j.1467-2979.2010.00356.x

242. L. Holsbeek *et al*., Heavy metals, organochlorines and polycyclic aromatic hydrocarbons in sperm whales stranded in the Southern North Sea during the 1994/1995 winter. *Mar. Pollut. Bull.* **38**, 304 (1999). doi:10.1016/S0025-326X(98)00150-7

243. P. Bustamante *et al*., Trace elements in two odontocete species (*Kogia breviceps* and *Globicephala macrorhynchus*) stranded in New Caledonia (South Pacific). *Environ. Pollut.* **124**, 263 (2003). doi:10.1016/S0269-7491(02)00480-3 Medline

244. T. Endo, O. Kimura, Y. Hisamichi, Y. Minoshima, K. Haraguchi, Age-dependent accumulation of heavy metals in a pod of killer whales (*Orcinus orca*) stranded in the northern area of Japan. *Chemosphere* **67**, 51 (2007). doi:10.1016/j.chemosphere.2006.09.086 Medline

245. N. A. Rudneva, N. M. Pronin, L. V. Rudneva, Microelements and heavy metals in the muscles of the muskrat (*Ondatra zibethica*) from the Selenga River Delta. *Russ. J. Ecol.* **36**, 435 (2005). doi:10.1007/s11184-005-0098-7

246. M. Kirchgessner, E. Grassmann, J. J. Kim, Fe- und Cu-Gehalte in Knochen, Muskel und Ganzkörper wachsender Ratten bei unterschiedlicher Fe- und Cu-Versorgung. *Z. Ernahrungswiss.* **23**, 20 (1984). doi:10.1007/BF02020893 Medline

247. J. C. Opazo, A. M. Sloan, K. L. Campbell, J. F. Storz, Origin and ascendancy of a chimeric fusion gene: the beta/delta-globin gene of paenungulate mammals. *Mol. Biol. Evol.* **26**, 1469 (2009). doi:10.1093/molbev/msp064 Medline

248. H. N. Poinar *et al*., Metagenomics to paleogenomics: Large-scale sequencing of mammoth DNA. *Science* **311**, 392 (2006). doi:10.1126/science.1123360 Medline

249. M. Bianchi *et al*., Recombinant expression of *Mus musculus* myoglobin. *Protein Expr. Purif.* **29**, 265 (2003). doi:10.1016/S1046-5928(03)00067-6 Medline