



Supplementary Materials for

Evolution of Mammalian Diving Capacity Traced by Myoglobin Net Surface Charge

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This PDF file includes:

Supplementary Text
Figs. S1 to S6
Tables S1 to S7
References

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 Erinacidae – 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*) – (*Lyacon, 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.

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(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,((((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,Bohead*: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):5.20000000):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:17.90000000):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,(Douroucoulis: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
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0000,((Siamang:10.30000000,(White_cheeked_gibbon:9.20000000,Agile_gibbon:9.20000000):1.10000000):11.20000000,(Orangutan:15.90000000,(Mountain_gorilla:10.70000000,(Chimpanzee:8.10000000,Human:8.10000000):2.60000000):5.20000000):5.60000000):7.80000000):13.50000000):28.70000000):11.80000000):8.70000000):9.30000000):88.70000000):27.80000000):145.50000000);

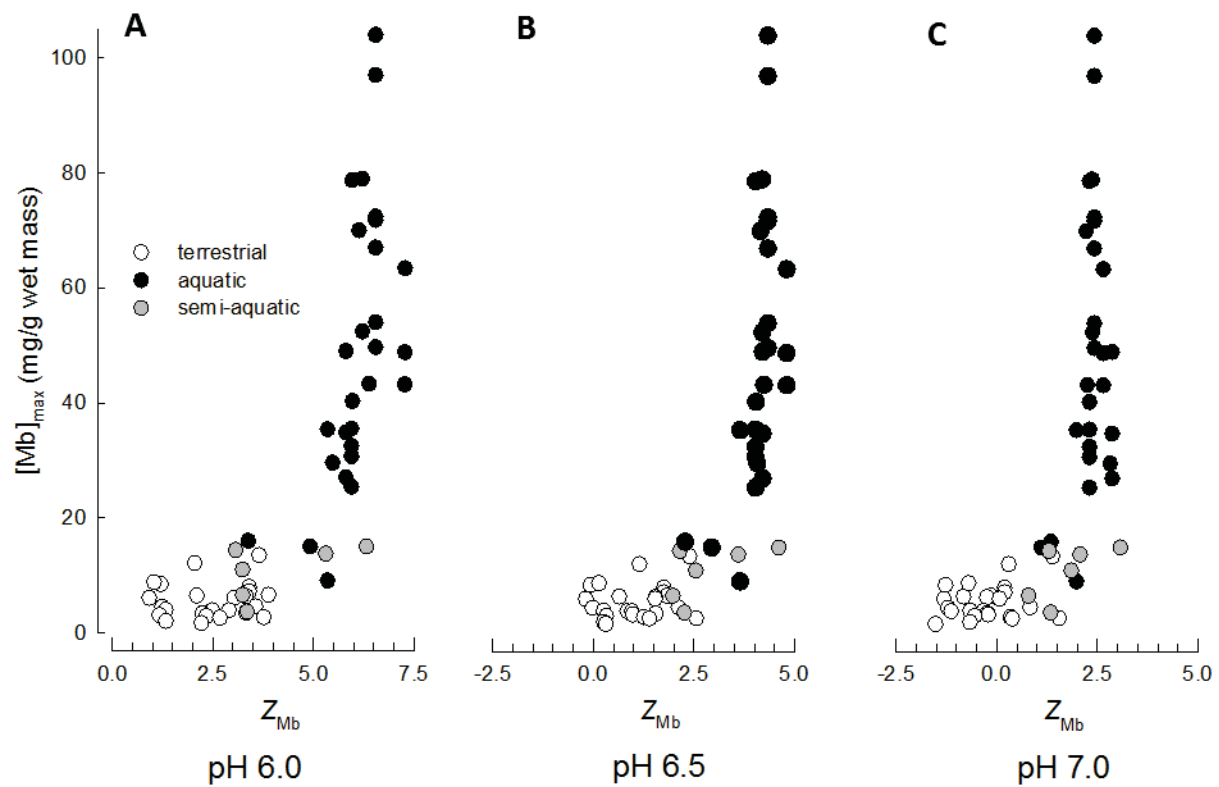
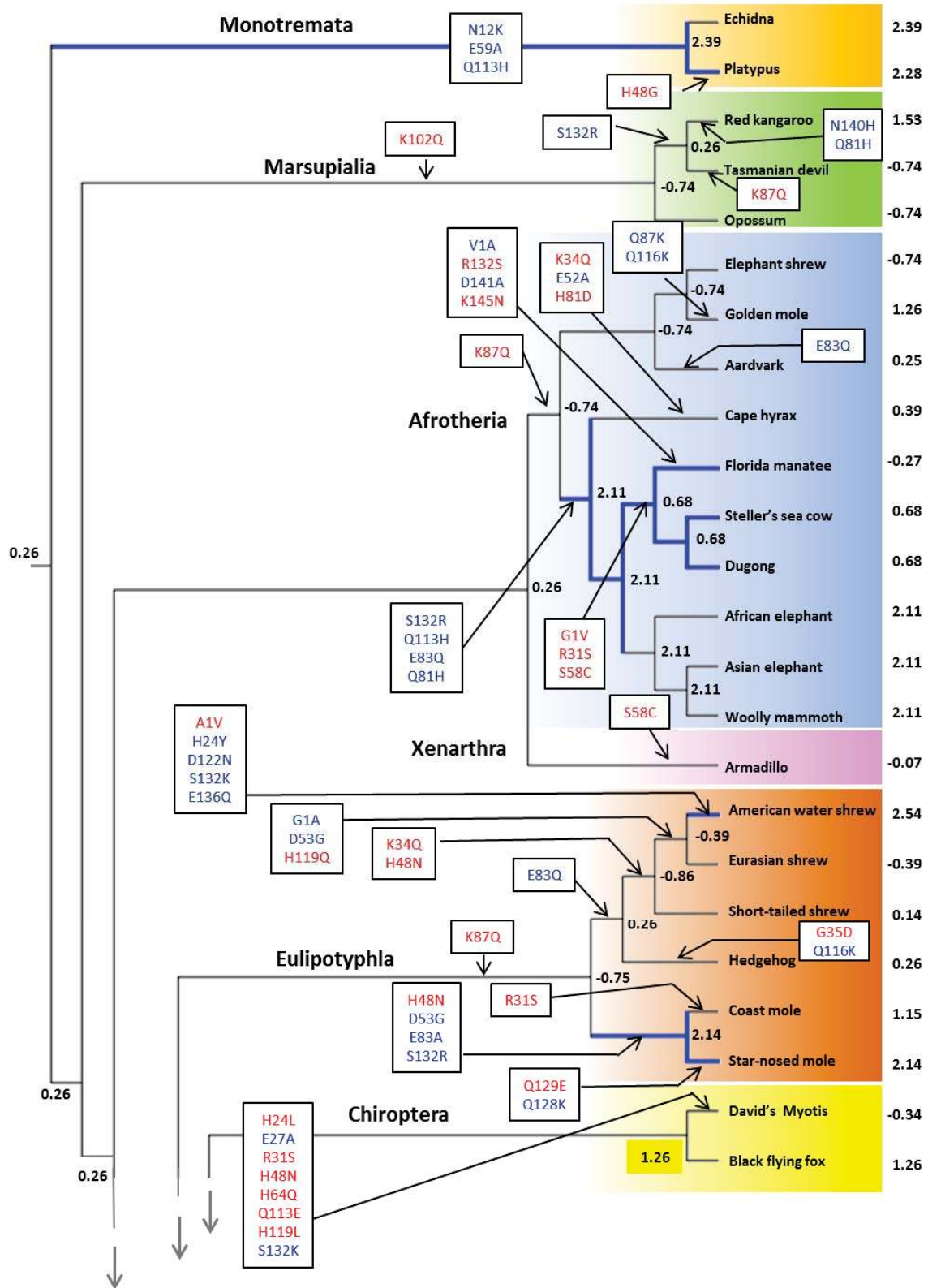


Fig. S1.

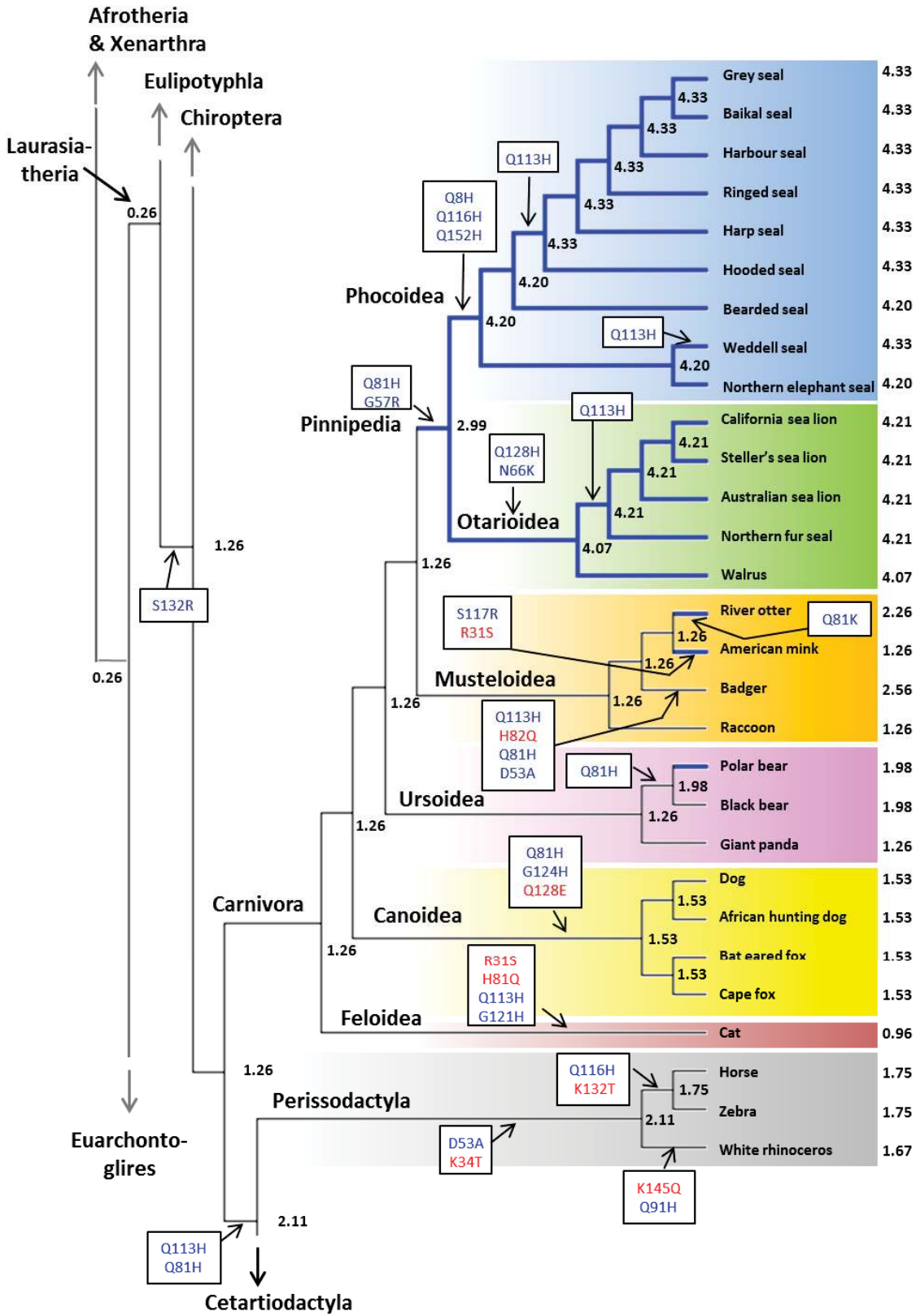
Mb net surface charge, Z_{Mb} , and maximal muscle concentration, $[Mb]_{max}$, in terrestrial, semi-aquatic and aquatic mammals. Z_{Mb} was obtained by modeling for a) pH 6.0, b) pH 6.5 and c) pH 7.0 (17).

(Fig. S2)



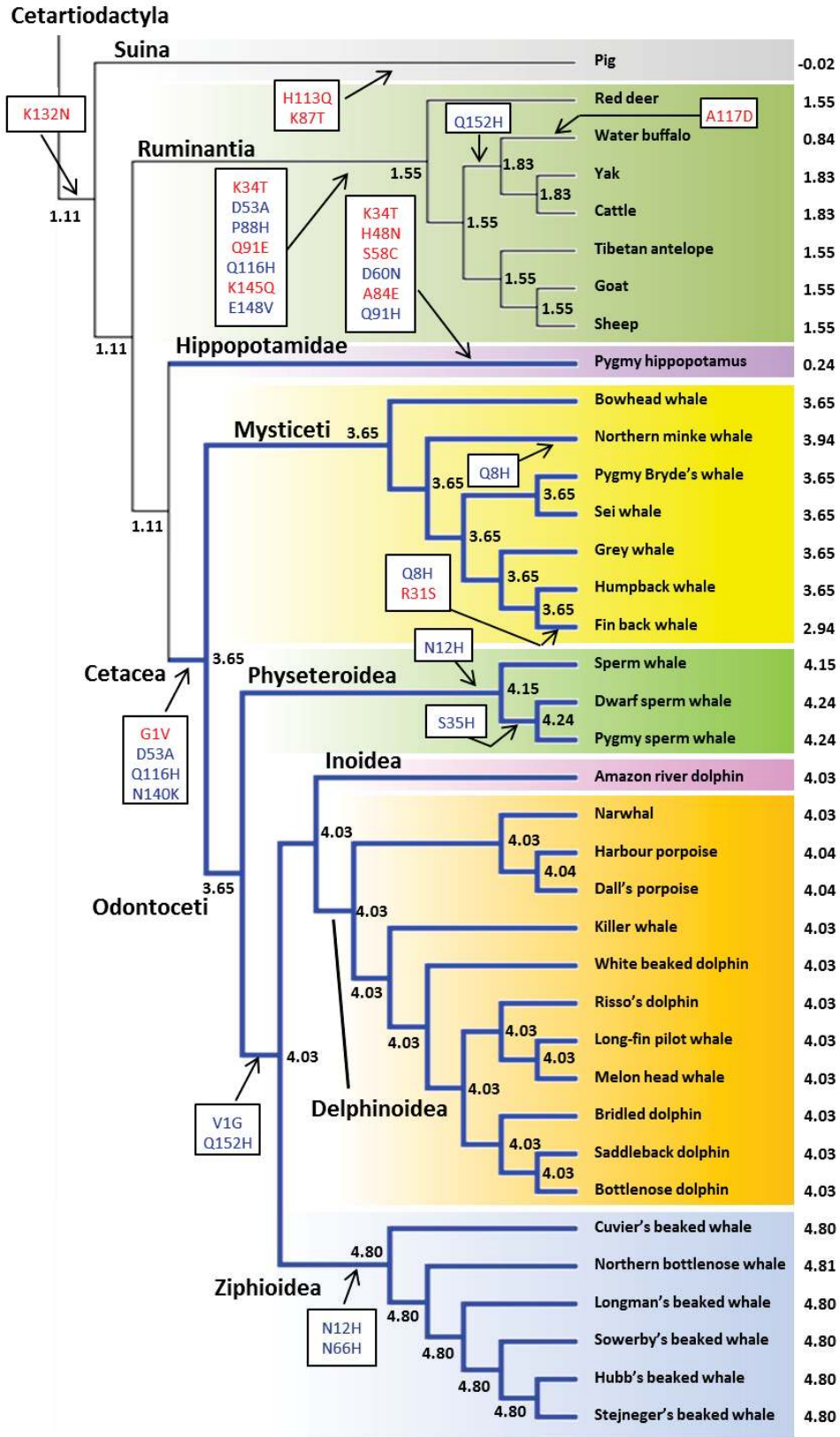
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(Fig. S2 continued)



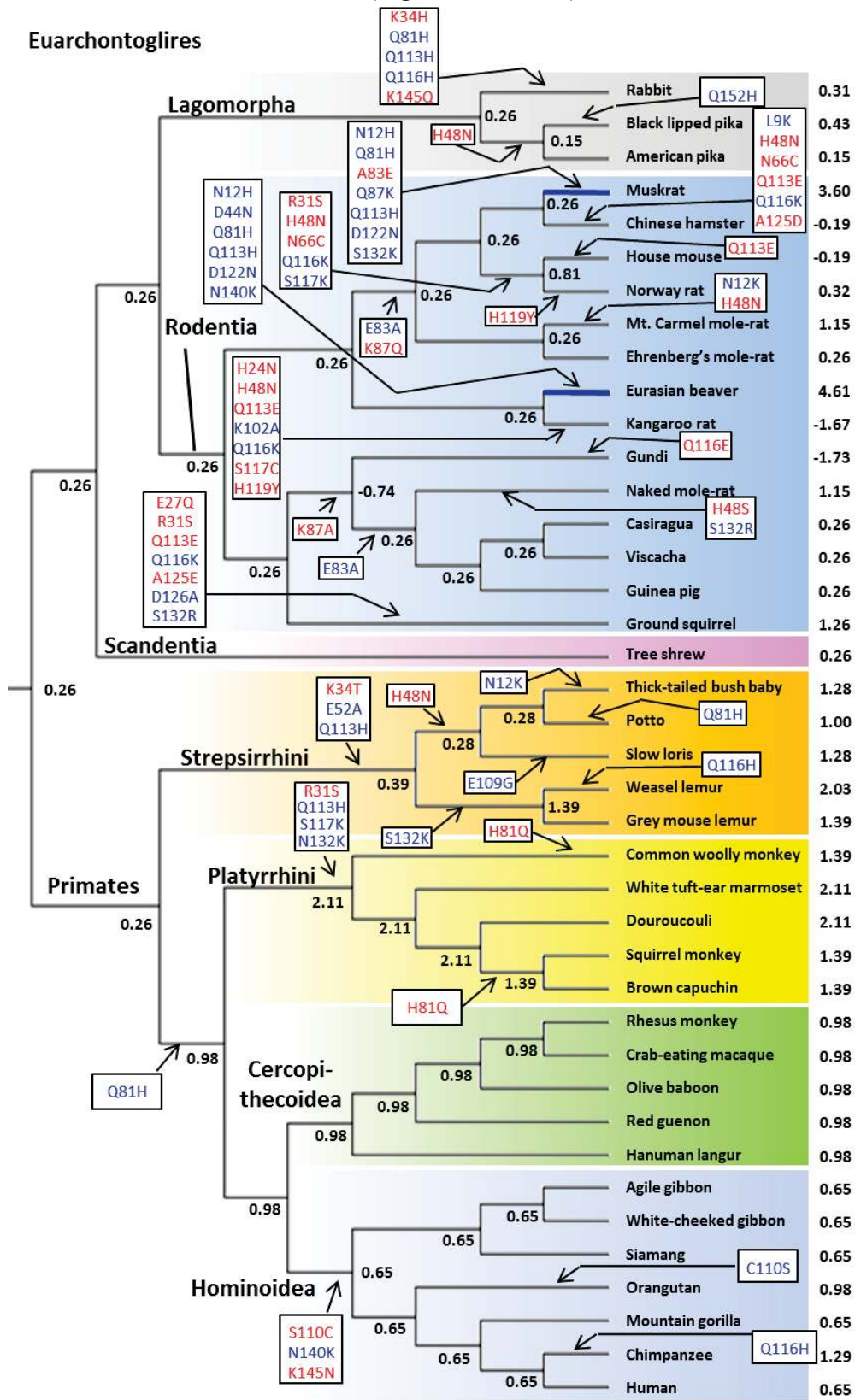
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(Fig. S2 continued)



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(Fig. S2 continued)



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(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.

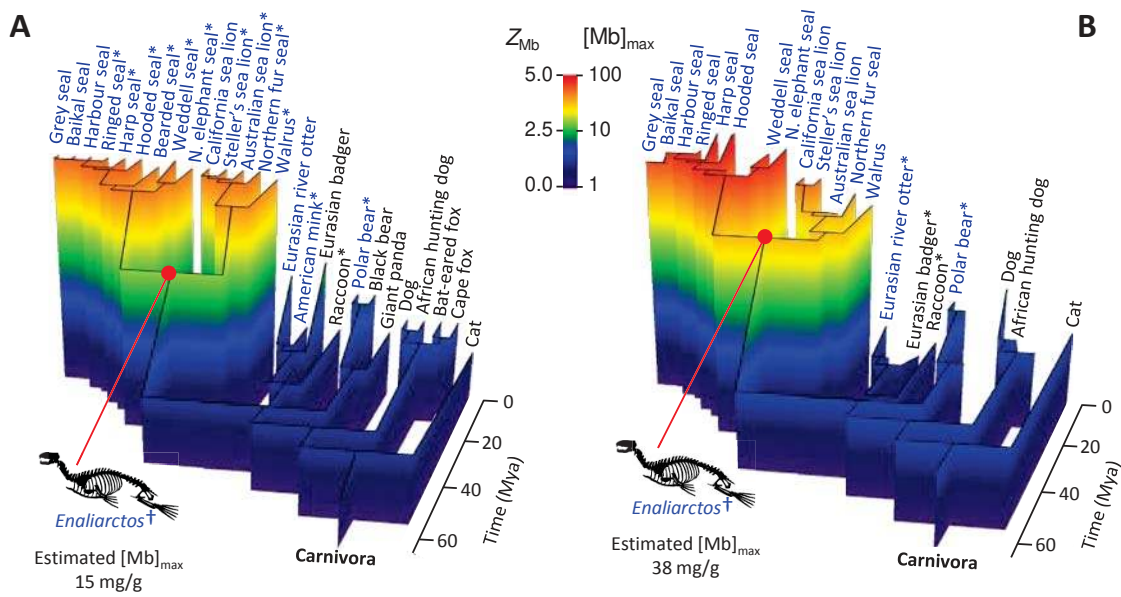
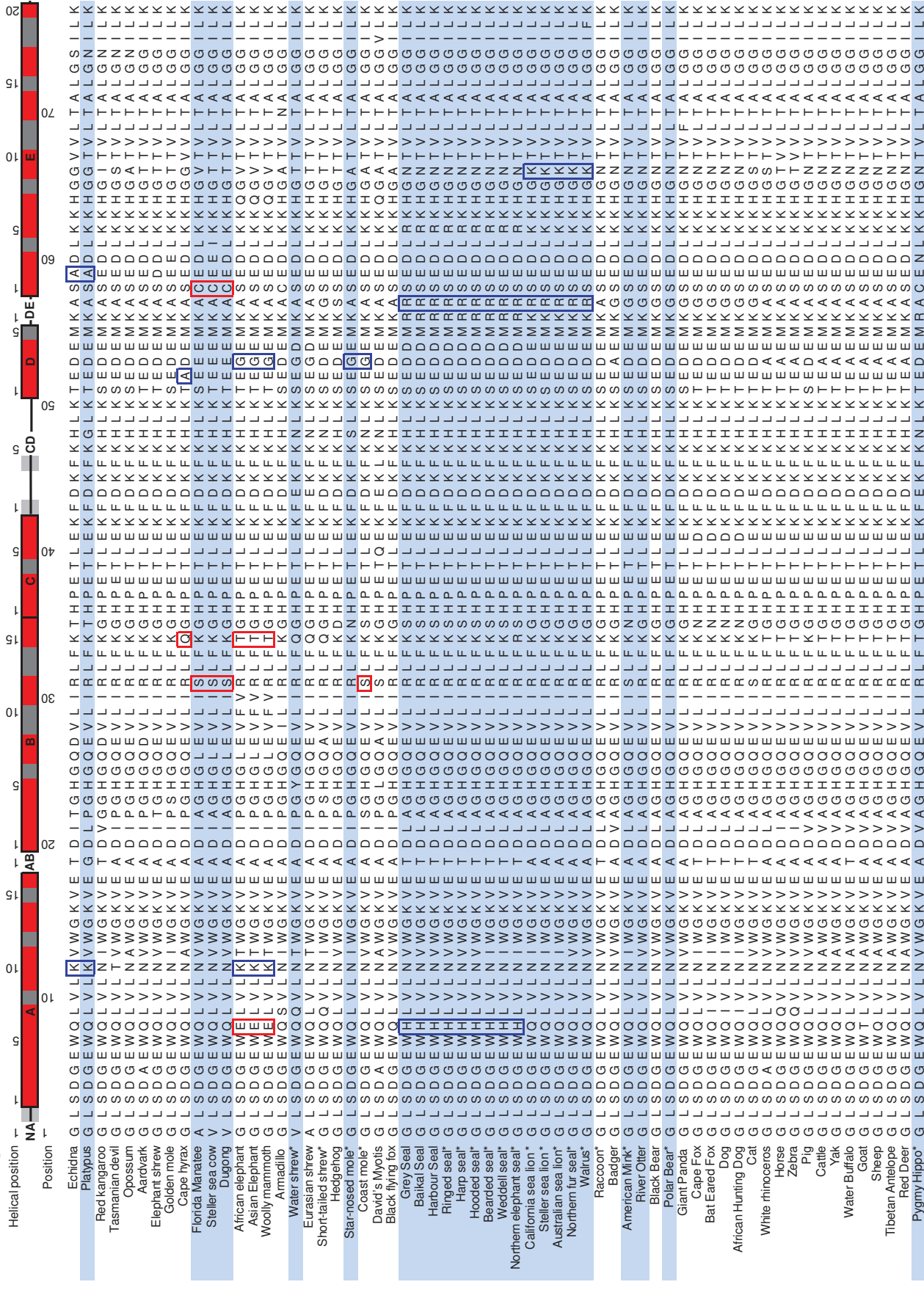


Fig. S3

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.

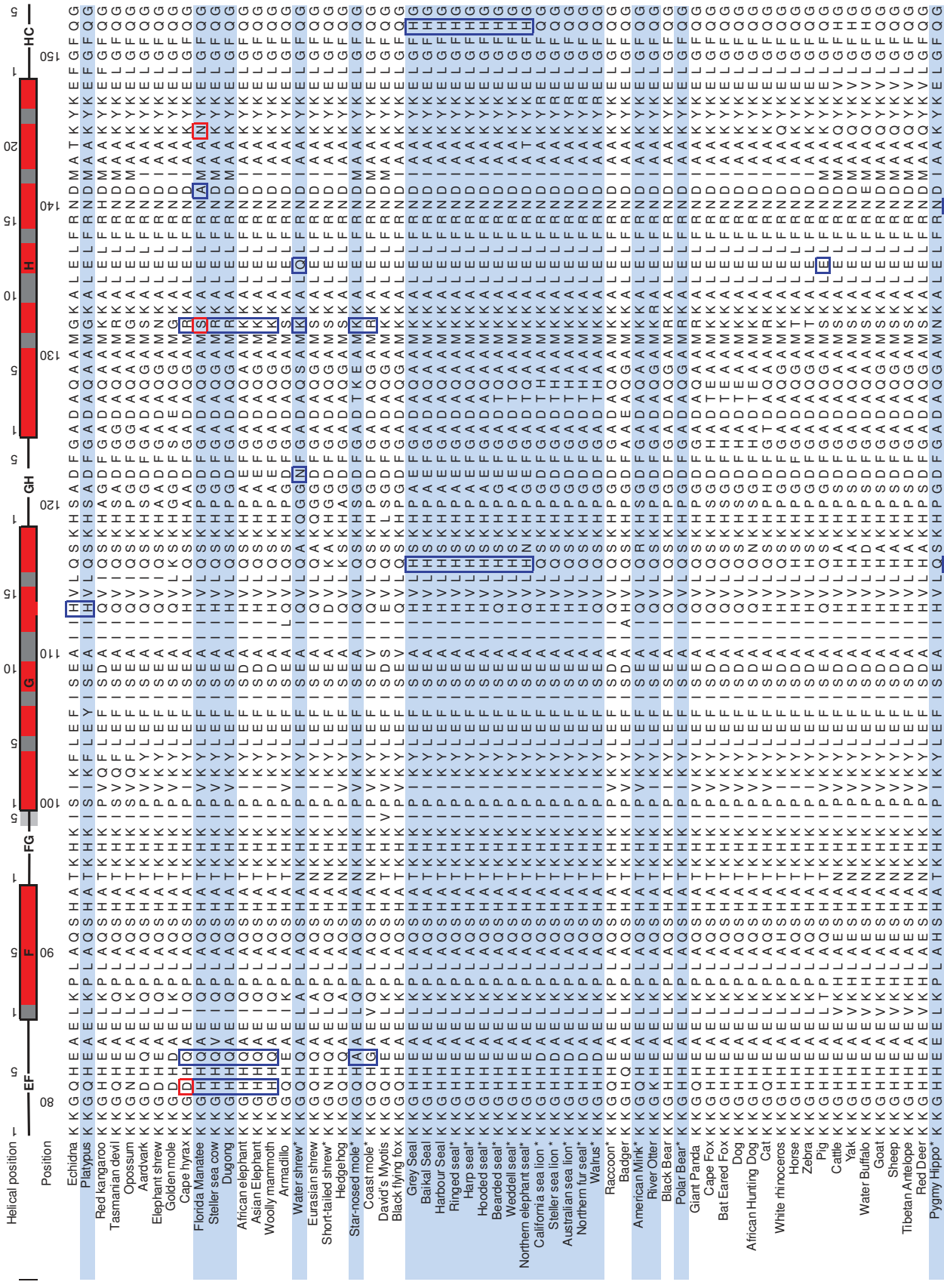
A (Fig. S4)



B (Fig. S4)

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

C (Fig. S4)



D (Fig. S4)

Fin Back Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Humpback Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Grey Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Pygmy Bryde's Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Sei Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Minkie Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Bowhead	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Pygmy Sperm Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Dwarf Sperm Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Sperm whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
teineger's Beaked Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Hubb's Beaked Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Sowerby's beaked whale*	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
ongmans' Beaked Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
lorthern bottlenose whale*	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Cuvier's beaked whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Amazon River Dolphin	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Narwhal*	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Harbour Porpoise	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Dall's Porpoise	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Killer Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
White beaked dolphin*	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Risso's Dolphin*	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Long-Fin Pilot Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Melon Head Whale	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Bridled dolphin	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Saddleback Dolphin	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Bottlenose Dolphin	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Rabbit	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Black Lipped Pika	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
American Pika	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
House Mouse	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Norway Rat	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
House Mouse	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Chinese hamster	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Muskrat	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Spalax carmeli	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Ehrenberg's Mole-Rat	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Kangaroo rat	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Eurasian Beaver	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Plains Viscacha	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Casiragua	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Guinea Pig	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Northern Gundi	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Tree Shrew	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Thick Tailed Bush Baby	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Potto	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE	FE	IS	DA	I	H	V	L	H	S	R	H	P	A	D	FG	AD	QA	AM	KA	LE	FR	ND	I	A	AK	YK	EL	GF	FG
Slow Loris	KK	GH	HE	AE	EL	KP	LA	QS	HA	TK	KK	IP	KY	LE</																													

(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.

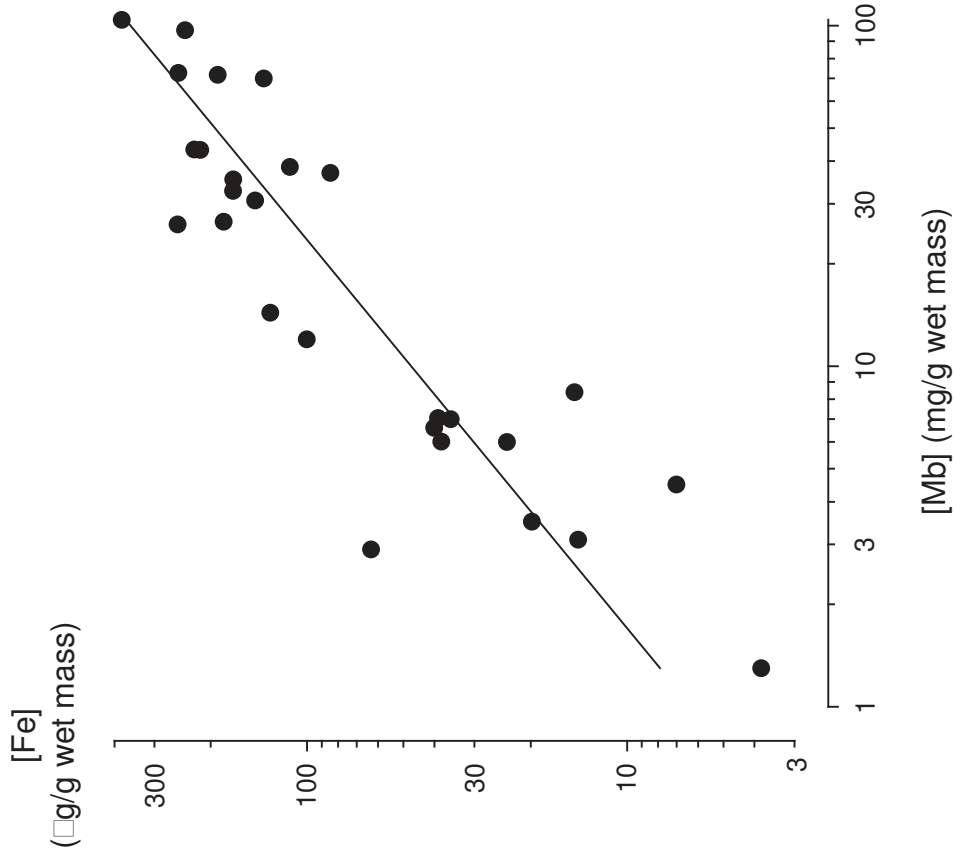


Fig. S5 Correlation between [Fe] and [Mb] in skeletal muscle of 27 mammalian diving and non-diving species. The linear regression line follows the equation $\log [\text{Fe}] = 0.877 \log [\text{Mb}] + 0.797$ ($r^2 = 0.786588$). See Tab. S4 for data and sources.

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

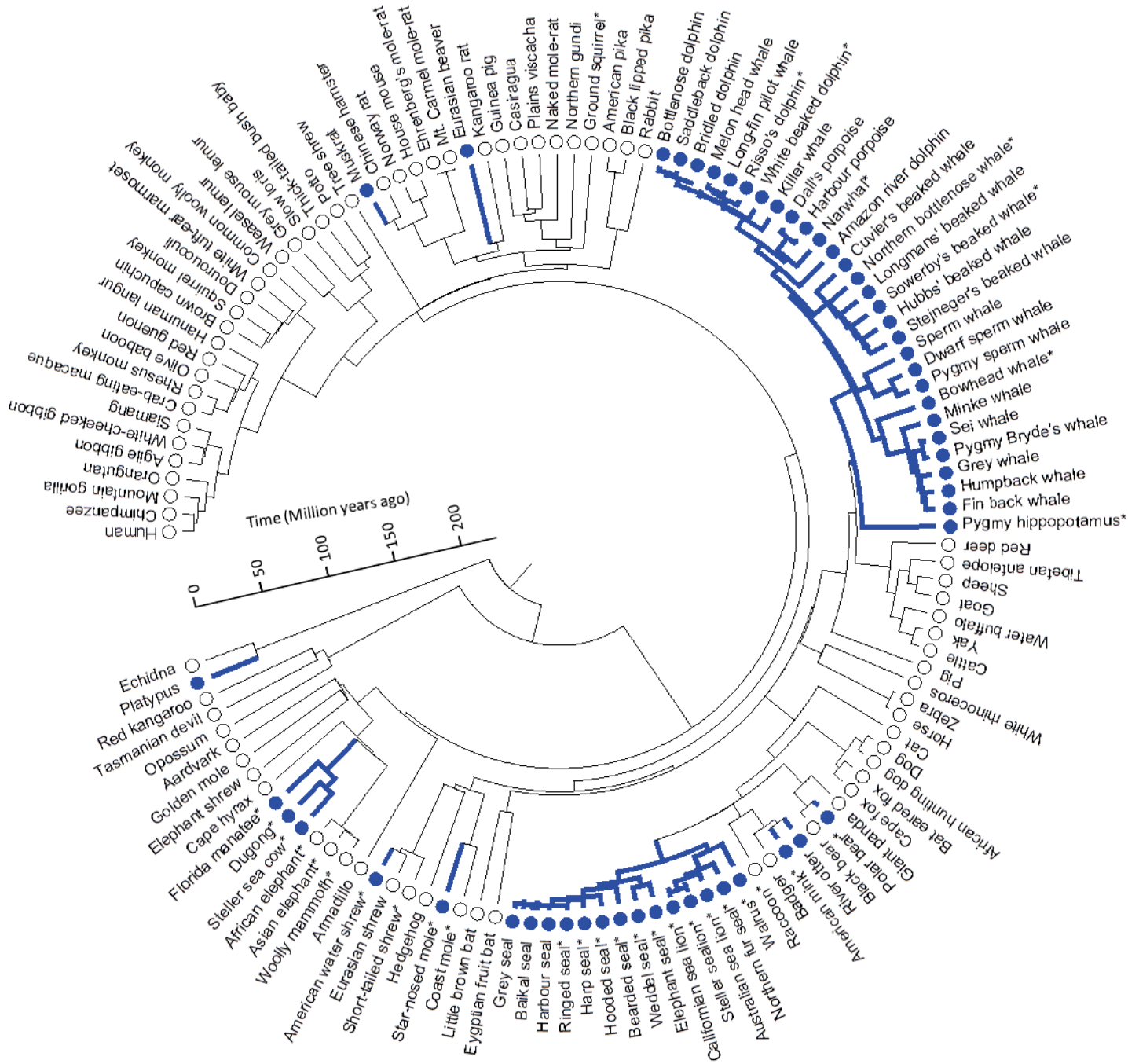


Table S1.

Maximal dive time (t_{\max}), body mass, maximal skeletal muscle Mb content ($[\text{Mb}]_{\max}$), 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)	$[\text{Mb}]_{\max}$ (mg/g wet mass)	Z_{Mb}	Accession No.	Habitat
Monotremata							
Echidna	<i>Tachyglossus aculeatus</i>			15.30 (33)	2.39	P02195	SF
Platypus	<i>Ornithorhynchus anatinus</i>	138 (91)	1.800 (91)	16.00 (92)	2.28	P02196	SA
Marsupialia							
Red kangaroo	<i>Macropus rufus</i>				1.53	P02194	
Tasmanian devil	<i>Sarcophilus harrisi</i>				-0.74	<u>ENSSHAT0000006888</u>	
Opossum	<i>Didelphis virginiana</i>				-0.74	P02193	
Placentalia							
Afrotheria							
Afroinsectiphilia							
Elephant shrew	<i>Elephantulus edwardii</i>				-0.74	AMGZ01134077	SA
Aquatic tenrec	<i>Limnogale mergulus</i>	15 (93)	0.105 (93)		1.26	AMDV01153116	F
Golden mole	<i>Chrysochloris asiatica</i>				0.25	ALYB01283710	SF
Aardvark	<i>Orycteropus afer</i>					(differs from previously published protein sequence P02164)	
Paenungulata							
Cape hyrax	<i>Procavia capensis</i>				0.39	<u>ENSPCAT0000003677</u>	
Amazonian manatee	<i>Trichechus inunguis</i>			9.45 (94)	-0.27	KC524769*	A
Florida manatee	<i>Trichechus manatus latirostris</i>	200 (95)	600.000 (96)			AHIN01036638.1	A
Steller's sea cow†	<i>Hydrodamalis gigas</i>	300 (48)	3423.000 (96)		0.68	KC524738* (specimen 1)	A
						KC524739* (specimen 2)	
						KC524740* (specimen 3)	
Dugong	<i>Dugong dugon</i>	240 (97)	300.000 (96)		0.68	KC524735*	A
						KC524736*	
African elephant	<i>Loxodonta africana</i>				2.11	KC524741*	
						AAGU03077599.1	

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

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

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

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

California vole	<i>Microtus californicus</i>	20	(200)	0.045	(201)	14.38	(202)	3.60	P32428	3.60	P32428	SA
Muskrat	<i>Ondatra zibethicus</i>	210	(202)	0.681	(202)			-0.19	EGW14348			SF
Chinese hamster	<i>Cricetulus griseus</i>											SA
Australian water rat	<i>Hydromys chrysogaster</i>	32	(203)	0.681	(204)	6.04	(113)	-0.19	NP_038621			F
House mouse	<i>Mus musculus</i>					3.60	(55)	0.32	NP_067599			F
Norway rat	<i>Rattus norvegicus</i>	20	(205)	0.300	(205)	4.00	(206)	1.15	CAL91965			F
Mt. Carmel mole-rat	<i>Spalax carmeli</i>							0.26	P04248			
Ehrenberg's mole-rat	<i>Nannospalax ehrenbergi</i>											
North American beaver	<i>Castor canadensis</i>	300	(207)	18.000	(207)	16.00	(208)					SA
Eurasian beaver	<i>Castor fiber</i>					15.61	b(209)	4.61	P14396			SA
Kangaroo rat	<i>Dipodomys ordii</i>							-1.67	<u>ENSDORT00000014499</u>			SF
Gundi	<i>Ctenodactylus gundi</i>							-1.73	P20856			
Naked mole-rat	<i>Heterocephalus glaber</i>							1.15	AFSB01158705 (exon 1)			F
									AFSB01158703 (exon 2 and 3)			
Casiragua	<i>Proechimys guairae</i>					0.26		0.26	P04249			
Viscacha	<i>Lagostomus maximus</i>					0.26		0.26	P04250			
Guinea pig	<i>Cavia porcellus</i>					6.09	(55)	0.26	<u>ENSCPOT000000006933</u>			HA
Coypu	<i>Myocastor coypu</i>	195	(210)	5.000	(211)							SA
Capybara	<i>Hydrochaeris hydrochaeris</i>	300	(98)	51.000	(98)							SA
Ground squirrel	<i>Ictidomys tridecemlineatus</i>							1.26	<u>ENSSTOT000000005611</u>			SF
Scandentia												
Tree shrew	<i>Tupaia glis</i>					0.26		0.26	P02165			
Primates												
Strepsirrhini												
Thick-tailed bush baby	<i>Otolemur crassicaudatus</i>					1.28		1.28	P02168			
Potto	<i>Perodicticus potto edwardsi</i>					1.00		1.00	P02166			
Slow loris	<i>Nycticebus coucang</i>					1.28		1.28	P02167			
Weasel lemur	<i>Lepilemur mustelinus</i>					2.03		2.03	P02169			
Grey mouse lemur	<i>Microcebus murinus</i>					1.39		1.39	<u>ENSMICT000000014099</u>			
Haplorrhini												
Platyrrhini												
Common woolly	<i>Lagothrix lagotricha</i>					1.39		1.39	P02154			

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	Estimated body mass (kg)	Comments	Image reference	Z_{Mb}	Rationale for Z_{Mb} assignment	$[Mb]_{max}$ (mg/g)	t_{max} (min)
Sirenia							
<i>Pezosiren portelli</i>	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							
<i>Moeritherium lyonsi</i>	810	Tapir-sized (35, 37)	(36)	4.11	based on the gain of two additional positive charges in the proboscidean lineage compared to the value (2.11) in the last common paen-ungulate ancestor of sirenians, hyraxes and proboscideans	26	13.7
Cetartiodactyla							
<i>Indohyus</i>							
	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
<i>Pakicetus attocki</i>	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
<i>Ambulocetus natans</i>							
	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
<i>Rodhocetus balochistanensis</i>							
	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
<i>Dorudon atrox</i>	2240	Based on vertebrae	(228)	3.65	similar to value at toothed whale-baleen whale split	21	13.7
<i>Basilosaurus cetoides</i>	6480	Based on occipital condyle breadth (229)	(230)	3.65	similar to value at toothed whale-baleen whale split	21	17.4
Pinnipedia							
<i>Enaliarctos mealsi</i>	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

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

Table S4.

Fe and Mb contents in mammalian skeletal muscle

Species	Scientific name	[Fe]($\mu\text{g/g}$ wet mass)	[Mb] (mg/g wet mass)
Carnivora			
Ringed seal	<i>Pusa (Phoca) hispida</i>	190.00 (161)	71.80 (113)
Harp seal	<i>Pagophilus groenlandicus</i>	240.00 (231)	97.00 (11)
Hooded seal	<i>Cystophora cristata</i>	379.00 (231)	104.00 (11)
Weddell seal	<i>Leptonychotes weddellii</i>	252.00 (232)	72.70 (121)
Northern fur seal	<i>Callorhinus ursinus</i>	113.00 (233)	38.50 (111)
Walrus	<i>Odobenus rosmarus</i>	170.00 (161)	32.75 (111)
Raccoon	<i>Procyon lotor</i>	63.00 (234)	2.90 a
Polar bear	<i>Ursus maritimus</i>	40.00 (235)	6.60 a
Perissodactyla			
Horse	<i>Equus caballus</i>	38.90 (236)	7.05 (156)
Cetartiodactyla			
Pig	<i>Sus scrofa</i>	7.00 (237)	4.50 (156)
Sika deer	<i>Cervus nippon</i>	38.00 (238)	6.01 (164)
Moose	<i>Alces alces</i>	30.00 (235)	No data
Cattle	<i>Bos taurus</i>	23.70 (237)	6.00 (99)
Sheep	<i>Ovis aries</i>	19.80 (237)	3.50 (163)
Bowhead whale	<i>Balaena mysticetus</i>	169.70 (239)	35.40 (10)
Northern minke whale	<i>Balaenoptera acutorostrata</i>	35.50 (232)	7.00 (168)
Fin back whale	<i>Balaenoptera physalus</i>	84.36 (240)	37.00 (176)
Blue whale	<i>Balaenoptera musculus</i>	14.58 (241)	8.40 (99)
Sperm whale	<i>Physeter macrocephalus</i>	136.36 (242)	70.00 (179)
Pygmy sperm whale	<i>Kogia breviceps</i>	224.63 (243)	43.30 (10)
Killer whale	<i>Orcinus orca</i>	145.00 (244)	30.70 (10)
Risso's dolphin	<i>Grampus griseus</i>	253.29 (240)	26.10 a
Bottlenose dolphin	<i>Tursiops truncatus</i>	181.84 (240)	35.34 (194)
Cuvier's beaked whale	<i>Ziphius cavirostris</i>	215.25 (240)	43.20 (10)
Lagomorpha			
Rabbit	<i>Oryctolagus cuniculus</i>	3.80 (237)	1.30 (199)
Rodentia			
Muskrat	<i>Ondatra zibethicus</i>	130.00 (245)	14.38 (202)
Norway rat	<i>Rattus norvegicus</i>	14.20 (246)	3.10 (246)
North American beaver	<i>Castor canadensis</i>	100.00 (234)	12.00 (208)
Eurasian beaver	<i>Castor fiber</i>	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†	<i>Hydrodamalis gigas</i>	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	<i>Dugong dugon</i>	Genomic DNA, female, Mabuiag Island, Torres Straits, Australia, previously extracted (247)	B. McDonald, James Cook University, Australia
Florida manatee	<i>Trichechus manatus</i>	Genomic DNA, female, Crystal River, Florida, USA, previously extracted (247)	R.K. Bonde, US Geological Survey, Gainesville, Florida
Woolly mammoth†	<i>Mammuthus primigenius</i>	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	<i>Elephas maximus</i>	Genomic DNA, male, previously extracted (247)	W. Korver, Bowmanville Zoo, Ontario
African elephant	<i>Loxodonta africana</i>	Genomic DNA, female, previously extracted (247)	W. Korver, Bowmanville Zoo, Ontario
Eulipotyphla			
Water shrew	<i>Sorex palustris</i>	Limb muscles, Nopiming Provincial Park, Manitoba, Canada	K. L. C.
Short-tailed shrew	<i>Blarina brevicauda</i>	Limb muscles, Whiteshell Provincial Park, Manitoba, Canada	K. L. C.
Star-nosed mole	<i>Condylura cristata</i>	Limb muscles, Nopiming Provincial Park, Manitoba, Canada	K. L. C.
Coast mole	<i>Scapanus orarius</i>	Limb muscles, Abbotsford, British Columbia,	K. L. C.

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

Hippopotamidae			
Pygmy hippo	<i>Choeropsis liberiensis</i>	Post mortem skeletal muscle sample of juvenile of unknown sex, Whipsnade Zoo, UK	E. Flach, Zoological Society London
Mysticeti			
Bowhead whale	<i>Balaena mysticetus</i>	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	<i>Balaenoptera acutorostrata</i>	Muscle from caudal peduncle of stranded juvenile male, Aug. 2008, Formby, Merseyside, UK	R. Deaville, Zoological Society London
Humpback whale	<i>Megaptera novaeangliae</i>	Longissimus dorsi of stranded juvenile male, Sep. 2009, River Thames, UK	R. Deaville, Zoological Society London
Odontoceti			
Narwhal	<i>Monodon monoceros</i>	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	<i>Lagenorhynchus albirostris</i>	Longissimus dorsi of stranded adult female, Cornwall, UK	R. Deaville, Zoological Society London
Risso's dolphin	<i>Grampus griseus</i>	Longissimus dorsi of stranded adult male, Sep. 2009, Anglesey, UK	R. Deaville, Zoological Society London
Northern-bottlenose whale	<i>Hyperoodon ampullatus</i>	Longissimus dorsi of stranded juvenile female, Sep. 2009, Dorset, UK	R. Deaville, Zoological Society London
Sowerby's beaked whale	<i>Mesoplodon bidens</i>	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	AGCTGTCTGGAGCCAGGAYAC
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	GAATGGGAGTTGGTGTGAA
Mp.Mb.Ex1.R2	GAACACCCTTGAACCTTTGA
Mp.Mb.Ex2.F1	CCTGGACTTGAACCAACCCT
Mp.Mb.Ex2.R1	TTCATCTCGCCCTCTGTCTT
Mp.Mb.Ex2.F2	AGGCTCTTACAGGTCATCC
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	YARTGYAGCCAACCTGGTCAAC
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

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