

SUPPLEMENTARY TABLES

Supplementary Table S1. Sources for cetacean rhodopsin sequences used in codon-based likelihood analyses

Common name	Binomen	Clade	Accession number
Cattle	<i>Bos taurus</i>	Cetruminantia	NM_001014890.1
Hippo	<i>Hippopotamus amphibius</i>	Whippomorpha	KC676928.1
Bowhead whale	<i>Balaena mysticetus</i>	Balaenidae (Mysticeti)	KC676921.1
N. Atlantic right whale	<i>Eubalaena glacialis</i>	Balaenidae (Mysticeti)	JQ730751.1
Pygmy right whale	<i>Caperea marginata</i>	Neobalaenidae (Mysticeti)	KC676926.1
N. Atlantic minke whale	<i>Balaenoptera acutorostrata acutorostrata</i>	Balaenopteridae (Mysticeti)	KC676922.1
N. Pacific minke whale	<i>Balaenoptera acutorostrata scammoni</i>	Balaenopteridae (Mysticeti)	XM_007192608.1
Blue whale	<i>Balaenoptera musculus</i>	Balaenopteridae (Mysticeti)	KC676923.1
Fin whale	<i>Balaenoptera physalus</i>	Balaenopteridae (Mysticeti)	KC676924.1
Sperm whale	<i>Physeter macrocephalus</i>	Physeteridae (Odontoceti)	XM_007126220.1
South-Asian river dolphin	<i>Platanista minor</i>	Platanistidae (Odontoceti)	KC676936.1
Sowerby's beaked whale	<i>Mesoplodon bidens</i>	Ziphiidae (Odontoceti)	AF055316.1
Baird's beaked whale	<i>Berardius bairdii</i>	Ziphiidae (Odontoceti)	KC676925.1
Cuvier's beaked whale	<i>Ziphius cavirostris</i>	Ziphiidae (Odontoceti)	KC676938.1
Yangtze river dolphin	<i>Lipotes vexillifer</i>	Lipotidae (Odontoceti)	XM_007461564.1
Franciscana	<i>Pontoporia blainvillei</i>	Pontoporidae (Odontoceti)	KC676937.1
Amazon river dolphin	<i>Inia geoffrensis</i>	Iniidae (Odontoceti)	KC676929.1
Beluga	<i>Delphinapterus leucas</i>	Monodontidae (Odontoceti)	KC676927.1
Finless porpoise	<i>Neophocaena phocaenoides</i>	Phocoenidae (Odontoceti)	KC676932.1
Harbour porpoise	<i>Phocoena phocoena</i>	Phocoenidae (Odontoceti)	KC676933.1
Dall's porpoise	<i>Phocoenoides dalli</i>	Phocoenidae (Odontoceti)	KC676934.1
Killer whale	<i>Orcinus orca</i>	Delphinidae (Odontoceti)	XM_004284305.1
Bottlenose dolphin	<i>Tursiops truncatus</i>	Delphinidae (Odontoceti)	AF055456.1
Pilot whale	<i>Globicephala melas</i>	Delphinidae (Odontoceti)	AF055315.1
Common dolphin	<i>Delphinus delphis</i>	Delphinidae (Odontoceti)	AF055314.1

Supplementary Table S2. Likelihood ratio tests for random-sites models (PAML) of the cetacean RH1 Bayesian gene tree derived from only synonymous nucleotide sites

Model	np	ln <i>L</i>	κ	Parameters ^a			Null	LRT	df	p
				ω_0/p	ω_1/q	ω_2/ω_p				
M0	49	-3178.83	4.17	0.12						
M1a	50	-3059.13	4.34	0.02 (87.9%)	1 (12.1%)					
M2a	52	-3052.65	4.45	0.02 (87.8%)	1 (11.1%)	5.60 (1.1%)	M1a	12.96	2	0.002*
M3	53	-3052.36	4.44	0.02 (87.2%)	0.86 (11.5%)	4.80 (1.3%)	M0	252.94	4	0.000*
M7	50	-3061.69	4.33	0.01	0.03					
M8a	51	-3059.02	4.33	0.34	13.63	1 (11.3%)				
M8	52	-3053.53	4.44	0.06	0.49	4.32 (1.5%)	M7	16.32	2	0.000*
							M8a	10.98	1	0.001*

Note: np, number of parameters; ln *L*, ln likelihood; κ , transition/transversion ratio; df, degrees of freedom. ^aFor models M0-M3, the ω values for each site class (ω_0 - ω_2) are shown with their proportions in parentheses. For models M7-M8, *p* and *q* describe the shape of the beta distribution, and ω_p refers to the positively selected site class (with proportion in parentheses) for models M8 and M8a (where it is constrained to one).

Supplementary Table S3. PARRIS test for positive selection (HYPHY) on the cetacean RH1 species tree

Model	$\ln L$	Parameters			LRT	p
		ω_1	ω_2	ω_3		
Null	-3006.79	0.01 (82.2%)	1 (17.8%)			
Positive selection	-3003.68	0.00 (81.1%)	1 (16.9%)	2.78 (2.0%)	6.22	0.043*

Supplementary Table S4. Results from branch-site model (PAML) analyses on the cetacean RH1 species tree

Partition ^a	Model	np	ln L	κ	Parameters ^b			AIC ^c	Null	LRT	df	p
					ω_0	ω_1	ω_2					
Cetacea	M2a_rel	52	-3047.29	4.3	0.02 (87.9%)	1.00 (11.6%)	7.13 (0.5%)	4.34				
	Brs_Null	51	-3047.42	4.3	B: 0.02	B: 1.00	B: 0.02	2.60	B: 1.00			
					F: 0.02 (86.8%)	F: 1.00 (5.8%)	F: 1.00 (6.9%)		F: 1.00 (0.4%)			
	Brs_Alt	52	-3047.2	4.3	B: 0.02	B: 1.00	B: 0.02	4.16	B: 1.00			0.507
					F: 0.02 (87.4%)	F: 1.00 (5.7%)	F: 1.24 (6.4%)		F: 1.24 (0.4%)			
	CmC	53	-3047.27	4.3	B: 0.02	B: 1.00	B: 0.10	6.30	M2a_rel	0.04	1	0.841
Odontoceti	Brs_Null	51	-3052.55	4.3	F: 0.02 (87.5%)	F: 1.00 (5.1%)	F: 1.26 (7.3%)	12.86	B: 1.00			
					B: 0.02	B: 1.00	B: 0.02		B: 1.00			
					F: 0.02 (87.7%)	F: 1.00 (11.2%)	F: 1.00 (0.9%)		F: 1.00 (0.1%)			
	Brs_Alt	52	-3049.99	4.3	B: 0.02	B: 1.00	B: 0.02	9.74	Brs_Null	5.12	1	0.024*
					F: 0.02 (87.7%)	F: 1.00 (11.6%)	F: 6.24 (0.5%)		F: 6.24 (0.1%)			
	CmC	53	-3047.28	4.4	B: 0.02	B: 1.00	B: 5.89	6.32	M2a_rel	0.02	1	0.888
Mysticeti	Brs_Null	51	-3052.82	4.3	F: 0.02 (87.9%)	F: 1.00 (11.2%)	F: 7.85 (0.9%)	13.40	B: 1.00			
					B: 0.02	B: 1.00	B: 0.02		B: 1.00 (0%)			
					F: 0.02 (88%)	F: 1.00 (12%)	F: 1.00 (0%)		F: 1.00 (0%)			
	Brs_Alt	52	-3052.82	4.3	B: 0.02	B: 1.00	B: 0.02	15.40	Brs_Null	0	1	1.000
					F: 0.02 (88%)	F: 1.00 (12%)	F: 1.00 (0%)		F: 1.00 (0%)			
	CmC	53	-3047.22	4.3	B: 0.02	B: 1.00	B: 7.33 (0.7%)	6.20	M2a_rel	0.14	1	0.708
Bath+Epi	Brs_Null	51	-3049.34	4.3	F: 0.02 (87.9%)	F: 1.00 (11.4%)	F: 2.29	6.44	B: 1.00			
					B: 0.02	B: 1.00	B: 0.02		B: 1.00			
					F: 0.02 (86.3%)	F: 1.00 (10.2%)	F: 1.00 (3.1%)		F: 1.00 (0.4%)			
	Brs_Alt	52	-3049.31	4.3	B: 0.02	B: 1.00	B: 0.02	8.38	Brs_Null	0.06	1	0.806
					F: 0.02 (86.8%)	F: 1.00 (10.2%)	F: 1.25 (2.6%)		F: 1.25 (0.3%)			
	CmC	53	-3046.38	4.3	B: 0.02	B: 1.00	B: 0.40	4.52	M2a_rel	1.82	1	0.177
Fresh	Brs_Null	51	-3054.04	4.3	F: 0.02 (87.9%)	F: 1.00 (5.7%)	F: 2.12 (6.4%)	15.84	B: 1.00			
					B: 0.02	B: 1.00	B: 0.02		B: 1.00			
					F: 0.02 (85.3%)	F: 1.00 (10.7%)	F: 1.00 (3.6%)		F: 1.00 (0.5%)			
	Brs_Alt	52	-3052.82	4.3	B: 0.02	B: 1.00	B: 0.02	15.40	Brs_Null	2.44	1	0.118
					F: 0.02 (87.3%)	F: 1.00 (11.0%)	F: 3.19 (1.5%)		F: 3.19 (0.2%)			
	CmC	53	-3047.22	4.3	B: 0.02	B: 1.00	B: 0.00	6.20	M2a_rel	0.14	1	0.708
					F: 0.02 (87.3%)	F: 1.00 (11.2%)		F: 2.41 (1.5%)				

^aPartitions listed indicate the foreground lineages. In all cases, the background partition contains the remaining taxa. ^b ω values for each site class are shown with the proportion of each in parentheses. B and F refer to the background and foreground partitions. ^cMinimum overall AIC (forging depth 3-partition of CmC: 6194.24) was used for all comparisons. Abbreviations np, number of parameters; lnL, ln likelihood; κ , transition/transversion ratio; df, degrees of freedom; Bath, bathypelagic; Epi, Epipelagic; Fresh, Freshwater/estuarine.

Supplementary Table S5. Assignment of species and internal nodes on the cetacean species tree to foraging depth and habitat partitions for clade model analyses

Node/terminus/clade	Assigned partition ^a	Source
Root Cetacea (Neoceti)	Mesopelagic, Oceanic	Ancestral neoceti morphology (Fordyce 2002a; Uhen 2008); diet/behaviour, dispersal (Uhen 2004; 2010); diving capacity (Mirceta et al. 2013)
Root Mysticeti	Mesopelagic, Oceanic	Ancestral odontocete and mysticete morphology (Uhen 2010; Marx 2012; Gatesy et al. 2013); dispersal (Steehan et al. 2009)
Root Odontoceti	Mesopelagic, Oceanic	Ancestral odontocete diet/behaviour (Lindberg et al. 2007; Johnston & Berta 2010)
Balaenidae	Epipelagic, Coastal	Ancestral balaenid morphology (Fordyce 2002b) Extant balaenid diet/behaviour, habitat (Curry and Brownell Jr. 2014)
<i>Caperea marginata</i> (Neobalaenidae)	Mesopelagic, Oceanic	Extant neobalaenid morphology, habitat (Kemper 2014)
Balaenopteridae	Mesopelagic, Oceanic	Ancestral balaenopterid morphology (Bisconti 2010) Extant balaenopterid diet/behaviour, habitat (Demere 2014)
<i>Physeter macrocephalus</i> (Physeteridae)	Bathypelagic, Oceanic	Ancestral physeterid morphology (Lambert et al. 2013a) Ancestral ziphiid morphology (Lambert et al. 2013b) Ancestral physeterid and ziphiid diving capacity (Mirceta et al. 2013) Extant physeterid diet/behaviour, habitat (Watwood et al. 2006)
Ziphiidae	Bathypelagic, Oceanic	Extant ziphiid diet/behaviour, habitat (Tyack et al. 2006)
<i>Platanista minor</i> (Platanistidae)	Epipelagic, Freshwater	Ancestral river dolphin morphology, independent freshwater invasions (Cassens et al. 2000; Hamilton et al. 2001; Geisler et al. 2011)
<i>Lipotes vexillifer</i> (Lipotidae)	Epipelagic, Freshwater	
<i>Pontoporia blainvillei</i> (Pontoporidae)	Epipelagic, Freshwater	
<i>Inia geoffrensis</i> (Iniidae)	Epipelagic, Freshwater	
Root Delphinoidea	Mesopelagic, Oceanic	Ancestral delphinoid morphology, behaviour, habitat (Kazar et al. 2014)
Root Monodontidae + Phocoenidae	Epipelagic, Coastal	Ancestral monodontid and phocoenid morphology and habitat (Velez-Juarbe & Pyenson 2012; Lambert 2008)
<i>Delphinapterus leucas</i> (Monodontidae)	Mesopelagic, Coastal	Extant monodontid diet/behaviour, habitat (Richard 2014)
Root Phocoenidae	Epipelagic, Coastal	Ancestral phocoenid morphology, habitat (Lambert 2008)
<i>Neophocaena phocaenoides</i>	Epipelagic, Coastal	Extant <i>N. phocaenoides</i> diet/behaviour, habitat (Jefferson 2014)
<i>Phocoenoides dalli</i>	Epipelagic, Oceanic	Extant <i>P. dalli</i> diet/behaviour, habitat (Jefferson 2014; Fajardo-Mellor et al. 2006)
<i>Phocoena phocoena</i>	Epipelagic, Coastal	Extant <i>P. phocoena</i> diet/behaviour, habitat (Jefferson 2014)
Root Delphinidae	Mesopelagic, Oceanic	Ancestral delphinid habitat (Banguera-Hinestroza et al. 2014) Ancestral delphinid morphology (Lindberg et al. 2007)
<i>Globicephala melas</i>	Mesopelagic, Oceanic	Extant <i>G. melas</i> diet/behaviour, habitat (Wang et al. 2014)
<i>Orcinus orca</i>	Epipelagic, Coastal	Extant <i>O. orca</i> diet/behaviour, habitat (Wang et al. 2014)
<i>Delphinus delphis</i>	Epipelagic, Oceanic	Extant <i>D. delphis</i> diet/behaviour, habitat (Wang et al. 2014)
<i>Tursiops truncatus</i>	Mesopelagic, Coastal	Extant <i>T. truncatus</i> diet/behaviour, habitat (Wang et al. 2014)

^aPartitions refer to foraging depth and habitat categories: Epipelagic (0-200 m), Mesopelagic (200 - 1000 m), Bathypelagic (>1000 m), Freshwater (rivers, lakes, estuaries), Coastal (within continental shelves), Oceanic (beyond continental shelves).

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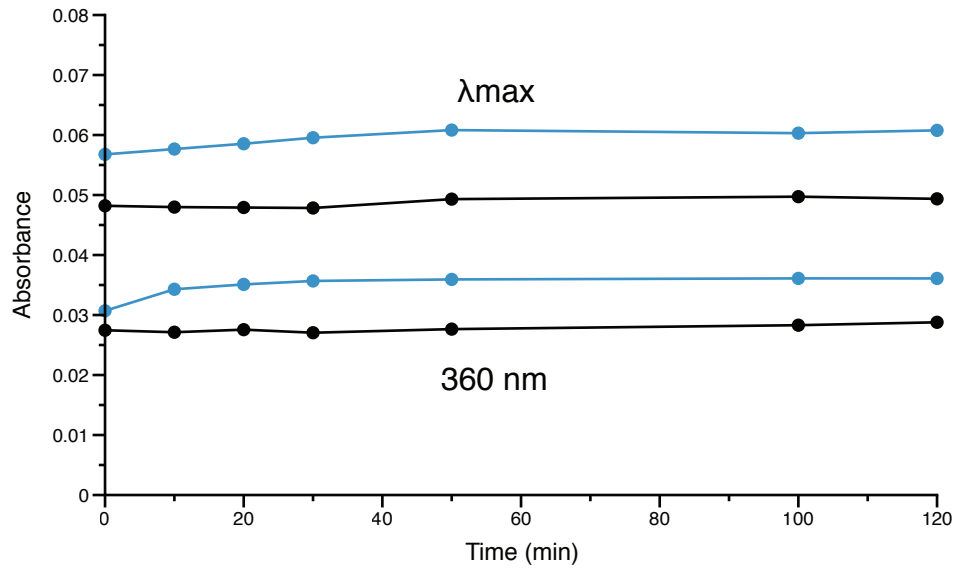


Fig. S1. Hydroxylamine (50 mM) exposure for wild-type bovine (black lines) and killer whale (blue lines) rhodopsins showing stability of the dark state peaks (487 nm, 499 nm) and retinal oxime peak (360 nm) over time.



Fig. S2. A) Cetacean rhodopsin Bayesian gene tree topology with mammalian outgroups. Bolded branches denote clades with positions that deviate from accepted species relationships for mammals (Springer et al. 2004; Bininda-Emonds et al. 2007) and cetaceans (McGowen 2011; Gatesy et al. 2013). In particular, members of Euarchonta and Glires, which are known to be monophyletic sister lineages, were spread paraphyletically among the basal branches. Within cetacea, Mysticeti was incorrectly nested within Odontoceti, *C. marginata* was incorrectly placed as the most basal mysticete, and *O. orca*, which is now known to be a basal delphinid (McGowen 2011), was not placed as such. Numbers at nodes indicate posterior probabilities.

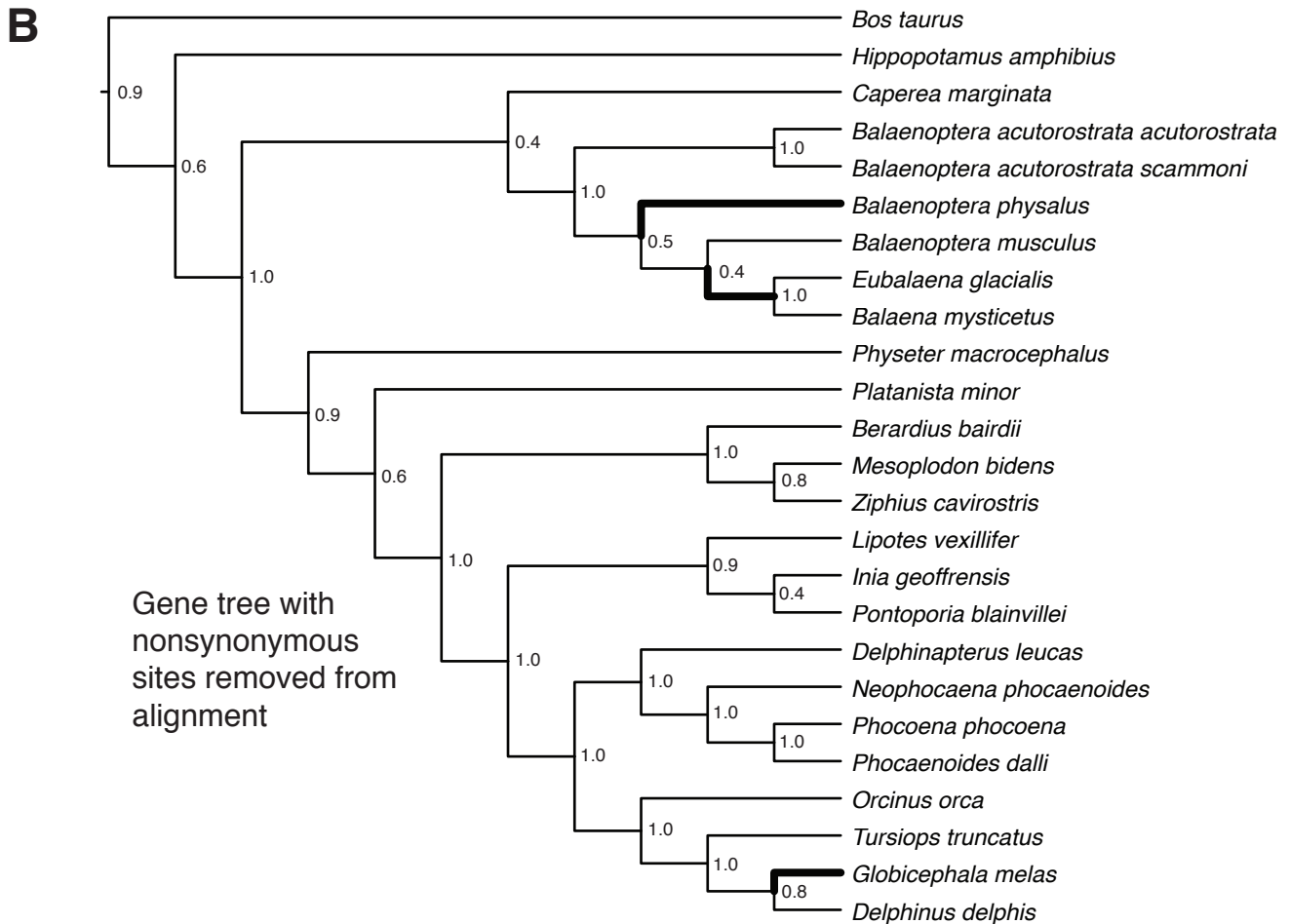


Fig. S2 cont. B) The pruned Bayesian topology that results when nonsynonymous sites are removed from the alignment (numbers at nodes indicate posterior probabilities). Bolded branches denote clades with positions that deviate from accepted species relationships for cetaceans (McGowen 2011; Gatesy et al. 2013). Removing nonsynonymous nucleotides (79 of 1059 nucleotides) from the alignment corrected the misplacement of Mysticeti within Odontoceti (*P. macrocephalus* and *P. minor* were no longer placed as basal cetaceans). Additionally, *O. orca* was moved to its proper position as a basal delphinid, but incorrect placements were still present among the mysticetes. Thus, overall, when nonsynonymous nucleotides were removed from the alignment, the gene tree was a considerably better match to the accepted species topology. Accession numbers for outgroups not listed in Supplementary Table S1 are as follows: JX103830.1, XM_001366188.2, NM_001280858.1, XM_004702378.1, AF055319.1, XM_004477246.1, U49742.1, NM_001082349.1, EF457995.1, XM_004902702.1, NM_001244407.1, NM_033441.1, XM_007517079.1, XM_006083811.1, NM_001009242.1, NM_001008276.1, AF055317.1, XM_004442424.1, AF008947.1, XM_004018534.1

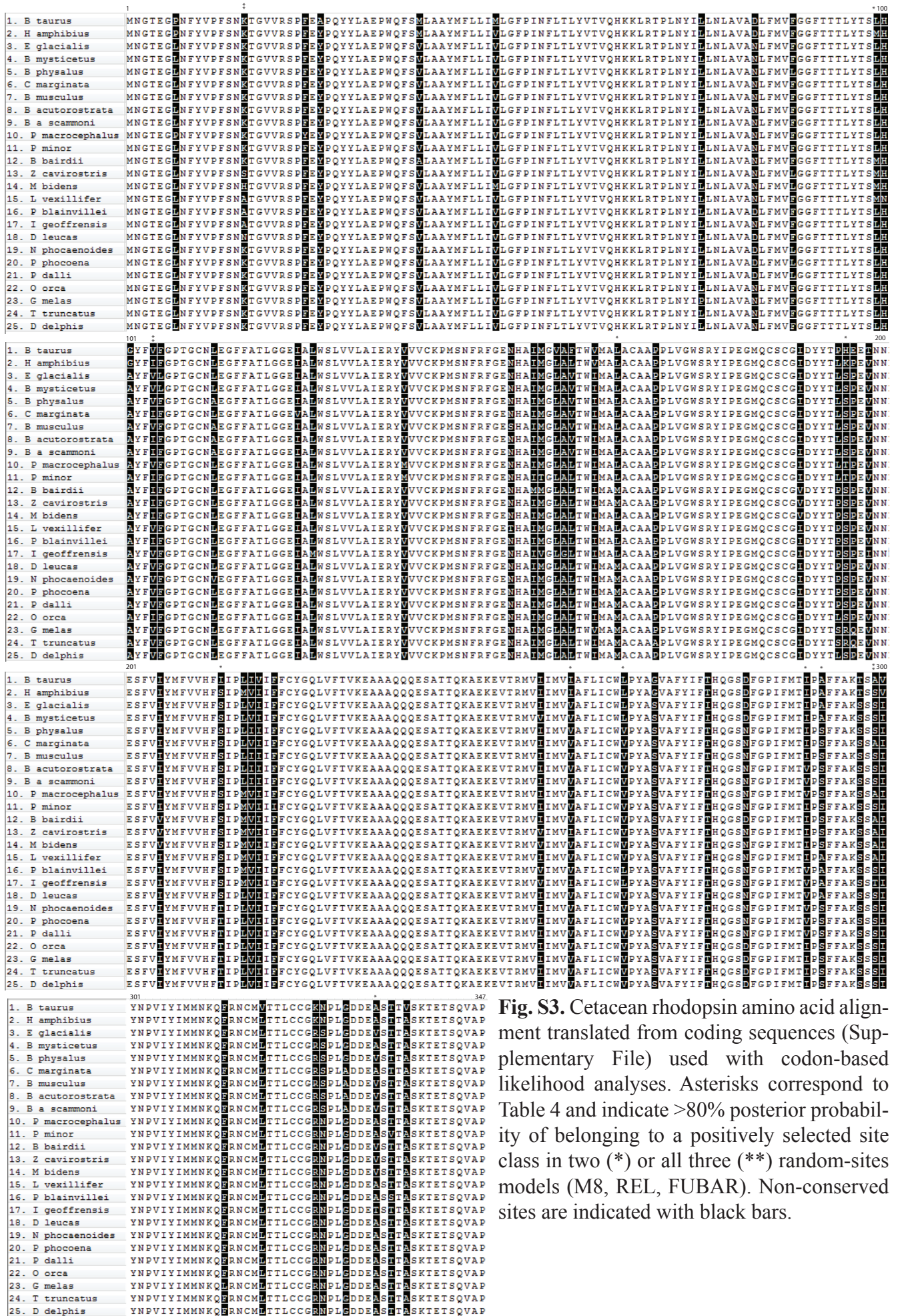


Fig. S3. Cetacean rhodopsin amino acid alignment translated from coding sequences (Supplementary File) used with codon-based likelihood analyses. Asterisks correspond to Table 4 and indicate >80% posterior probability of belonging to a positively selected site class in two (*) or all three (**) random-sites models (M8, REL, FUBAR). Non-conserved sites are indicated with black bars.