

The evolutionary history of cetacean brain and body size

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35 **Abstract**

36 Cetaceans rival primates in brain size relative to body size and include species with the largest
37 brains and biggest bodies to have ever evolved. Cetaceans are remarkably diverse, varying in
38 both phenotypes by several orders of magnitude, with notable differences between the two extant
39 sub-orders, Mysticeti and Odontoceti. We analyzed the evolutionary history of brain and body
40 mass, and relative brain size measured by the encephalization quotient (EQ), using a dataset of
41 extinct and extant taxa to capture temporal variation in the mode and direction of evolution. Our
42 results suggest that cetacean brain and body mass evolved under strong directional trends to
43 increase through time, but decreases in EQ were widespread. Mysticetes have significantly lower
44 EQs than odontocetes due to a shift in brain:body allometry following the divergence of the
45 suborders, caused by rapid increases in body mass in Mysticeti and a period of body mass
46 reduction in Odontoceti. The pattern in Cetacea contrasts with that in Primates, which
47 experienced strong trends to increase brain mass and relative brain size, but not body mass. We
48 discuss what these analyses reveal about the convergent evolution of large brains, and highlight
49 that until recently the most encephalized mammals were odontocetes, not primates.

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58 **Introduction**

59 Cetaceans, together with primates, have reached the upper range of mammalian brain size.
60 Cetacea include species with the largest brains to have ever evolved and species that rival
61 anthropoid primates for brain size relative to body size, superseded only by our own species
62 (Marino 1998). How and why their large brains evolved, what cognitive abilities they possess,
63 and what the convergent evolution of large brains reveals about the evolution of the human brain
64 are questions of considerable interest (Jerison 1973; Marino 1996) and substantial debate
65 (Manger 2006; Marino et al. 2007, 2008).

66 The encephalization quotient (EQ) quantifies variation in brain mass not explained by the
67 allometric relationship between brain and body mass (Jerison 1973). Variation in mammalian
68 EQ, or other measures of relative brain size, is associated with factors such as diet (Clutton-
69 Brock and Harvey 1980), social behavior (Sawaguchi 1992; Dunbar 1992), physical ecology
70 (Mace et al. 1981; Schultz and Dunbar 2006) and sensory specializations (Barton et al. 1995).
71 The original conception of EQ aimed to establish a comparative measure of cognitive ability
72 among species (Jerison 1973), and it has long been assumed that EQ tended to increase through
73 time during mammalian evolution (Jerison 1973; Gould 1988). This progressive view has been
74 challenged (Deacon 1990), and evolutionary trends in relative brain size may be limited to
75 particular clades (Schultz and Dunbar 2010). It is also clear that brain structure can evolve
76 independently of overall changes in brain size (Barton and Harvey 2000) and that shifts in brain
77 architecture are related to ecology (de Winter and Oxnard 2001). Some authors have argued that
78 particular regions of the brain (Reader and Laland 2002), or neuron number irrespective of brain
79 or body mass (Herculano-Houzel 2011), are better predictors of cognitive ability. Undoubtedly
80 both specialization of individual brain components and co-evolution among functionally

81 connected structures play major roles in behavioural evolution (Barton 2012). Yet, despite being
82 a somewhat crude measure, there is evidence linking whole brain size to cognitive performance
83 (Deaner et al. 2007; Reader et al. 2011) and survival in novel environments (Sol et al. 2008).
84 Although non-cognitive hypotheses have been proposed to explain the large brains of
85 odontocetes (Manger 2006), the consensus is that large brain size is evidence of, or necessary
86 for, the behavioural complexity and cognitive abilities observed in cetaceans (Marino 2002;
87 Simmonds 2006; Connor 2007; Marino et al. 2007).

88 Primates is one order that shows a strong trend for directional increases in relative brain
89 size (Montgomery et al. 2010). Increases in relative brain size are almost ubiquitous across the
90 primate tree and are produced by directional evolution of increased brain mass in a background
91 of body mass evolution that shows no significant trend to increase through time (Montgomery et
92 al. 2010). This suggests that brain and body mass evolution has become developmentally
93 decoupled in primates (Lande 1979) and that selection may have acted on the developmental
94 mechanisms controlling brain mass (Montgomery et al. 2011; Montgomery and Mundy 2012).
95 Whether or not the same pattern of evolutionary history and developmental mechanisms are
96 relevant to cetacean brain evolution is not known. A comparison between the evolution of
97 encephalization in primates and cetaceans provides an opportunity to identify shared and clade-
98 specific factors contributing to the evolution of large brains in two orders which differ widely in
99 ecology, anatomy and evolutionary history (Marino 1996, 1998).

100 The distribution of EQ values across cetaceans suggests that high levels of
101 encephalization have evolved convergently multiple times (Marino 1998; Marino et al. 2004),
102 but previous tests for directional expansion of cetacean EQ have produced conflicting results
103 (Marino et al. 2004; Schultz and Dunbar 2010) and have not explored the relationship between

104 brain and body mass evolution. This is of particular interest given evidence that brain:body
105 allometry in cetaceans differs significantly from other mammals (Manger 2006; Boddy et al.
106 2012) and the high discrepancies in EQ between the extremely large bodied mysticetes, as
107 compared to most smaller bodied odontocetes (Marino 2004; Tartarelli and Bisconti 2006).
108 Shifts in brain:body scaling towards a lower allometric slope may reflect altered selection
109 pressures shaping one or both traits.

110 Body mass is likely to be an important adaptive trait in all cetaceans. Comparative
111 analyses suggest that selection on cetacean body mass is related to niche partitioning and diet,
112 which may have played an important role early in cetacean evolution (Slater et al. 2010; but see
113 also Pyenson and Sponberg 2011). Furthermore, the rate of body mass evolution in cetaceans far
114 exceeds that of terrestrial mammals (Evans et al. 2012) plausibly due to shifts in constraints on
115 body mass due to ‘aquatic weightlessness’ (Marino 1998; Huggenberger 2008), an abundant,
116 nutritious diet (Evans et al. 2012) and selection related to thermoregulation, predator defense,
117 migratory behavior, and feeding ecology (Millar and Hickling 1990; Noren and Williams 2000;
118 Fitzgerald 2006; Demere et al. 2008; Clauset 2013). Given the expected developmental
119 correlation between brain and body size (Atchley et al. 1984; Riska and Atchley 1985)
120 understanding how cetacean specific selective regimes on body mass have impacted brain
121 evolution is important for understanding the evolution of relative brain size. Although there is
122 some evidence that brains and bodies can evolve independently (Lande 1979; Gonzalez-Voyer et
123 al. 2009; Montgomery et al. 2010, 2011) whether this is generally the case, or if it is necessary
124 for the evolution of high levels of encephalization, is not clear.

125 In this study we analyze the evolution of brain mass, body mass, relative brain size and
126 the relationships among these traits. We test for macroevolutionary trends, reconstruct ancestral

127 phenotypes and perform a rigorous analysis of the effects of including fossil data in comparative
128 studies. We address a number of debated aspects of cetacean evolution including whether or not
129 relative brain size has increased through time (Marino et al. 2004; Schultz and Dunbar 2010),
130 whether shifts in EQ are generally due to changes in brain or body mass (Marino et al. 2004),
131 and how allometric shifts in the brain:body mass relationship have influenced the evolution of
132 relative brain size (Manger 2006; Boddy et al. 2012). Through a comparison with primates, our
133 results offer insights into the convergent evolution of large brains in Mammalia.

134

135 **Materials & methods**

136 **Phenotypic data**

137 Datasets of body mass and brain mass/endocranial volume (ECV) for extant and extinct
138 Cetacea were compiled from published sources (Table S1). Often species data were presented
139 without sample size or gender; we therefore took means of male and female values when
140 presented separately to standardize error introduced by merging data from multiple sources. Data
141 on ECV and brain mass from the same species showed negligible differences (OLS regression: p
142 < 0.001 , $ECV = 0.995[mass] + 0.011$) with the slope not significantly different to one ($p = 0.490$)
143 and the intercept not significantly different to zero ($p = 0.568$), and we therefore assumed a
144 $1g/cc^3$ relationship in species for which only volumetric data were available. To avoid the
145 inclusion of juveniles we took two precautions; where multiple datasets were available for a
146 species, data were taken from the source reporting the largest body mass, and we regressed body
147 mass estimates from the final dataset against maximum body masses reported in Reeves et al.
148 (2002) to identify outliers, which were then excluded. Data for extinct species were excluded if
149 the specimen was identified as a juvenile. Where estimates of body mass for extinct taxa differed

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150 between sources, the largest estimate was taken. Relative brain size was measured following
151 Jerison (1973):

$$152 \quad EQ = \text{Brain mass [g]} / (0.12 * \text{Body mass [g]}^{0.67})$$

153 All phenotypic data were \log_{10} -transformed to improve normality. As we are interested in
154 assessing the interplay between brain and body size through time, we refrained from adding
155 species for which body mass data exist but brain mass data do not as this would yield
156 incomparable metrics that could not be interpreted in a rigorous statistical framework.

157 In total our dataset includes 42 extant species, ~48% of living species, and 20 extinct
158 species, ~4% of valid extinct species (Supplementary Online Material). The latter value is
159 difficult to gauge; it is likely deflated by counting “valid” species which upon re-evaluation will
160 likely prove to be *nomen dubia*, but the discovery of new fossils could render it an
161 underestimation. A more relevant measure of coverage is the phylogenetic distribution of
162 samples. In this regard our data set includes 13/14 extant families (93%) and our fossil taxa span
163 a range of key taxonomic transitions. It is difficult to quantify the proportion of extinct families
164 sampled due to a lack of taxonomic information. Our dataset includes the smallest and largest
165 extant genera and a range of extinct body sizes. To begin to explore the stability of our results to
166 variation in sampling, we conducted a series of analyses examining the effects of excluding key
167 taxa the overall effects of including fossils, and the ability of our model to estimate known
168 species values. The results are stable to the inclusion/exclusion of different extinct taxa and
169 suggest the approach taken is robust (Supplementary Online Material).

170

171 **Phylogeny**

172 The phylogenetic hypothesis for extant and extinct species is a composite of published
173 molecular (McGowen et al. 2009; McGowen 2011) and morphological trees (Geisler et al. 2011).
174 To construct this phylogenetic framework, we started with a time-calibrated molecular tree
175 (McGowen et al. 2009) and adjusted for relationships within Delphinidae (McGowen 2011). A
176 relaxed clock analysis established divergence points among extant species (see Supplementary
177 Online Material). This composite timetree formed the scaffold on which to position extinct taxa.
178 Geisler et al. (2011) conducted a similar analysis and the scaffold they employed is consistent
179 with the calibrated molecular tree of the present study, so extinct taxa were positioned as in
180 Geisler et al. (2011). Additional taxa not included in that study, but for which brain and body
181 mass estimates are available, were placed on this tree based on three morphological, cladistic
182 analyses (Uhen 2004; Lambert 2005; Lambert et al. 2010; see Supplementary Online Material).

183 One challenging issue when incorporating fossils into our analysis is the length of
184 branches leading to, or subdivided by, extinct taxa. Data on the age of the specimens from which
185 the phenotypic data were compiled, and the first appearance of that species or related clade, were
186 used to constrain splitting events and branch lengths of terminal branches leading to extinct taxa.
187 Subdivision of an internal branch bisected by a branching event with an extinct species/clade is
188 more difficult. In the absence of a data driven way to subdivide such branches, we consistently
189 applied a rule throughout the tree which placed branch-splitting events so that the branches on
190 the extant tree were divided into branches of equal duration. To prevent extinct taxon from
191 having an undue influence on our reconstruction of ancestral states, when necessary, we pushed
192 divergences involving fossils back so that terminal branches leading to fossils and internal
193 branches immediately basal to these fossils were each ≥ 0.5 Ma (Figure 1). Additional
194 information on the construction of the phylogenetic hypothesis is given in the Supplementary

195 Online Material. We refer to “crown Cetacea” as including the common ancestor of Mysticeti
196 and Odontoceti, and “stem Cetacea” as all extinct cetaceans that diverged before the last
197 common ancestor of Mysticeti and Odontoceti (Figure 1). A similar system is used when
198 referring to Odontoceti.

199

200 **Evolutionary analyses and ancestral state reconstructions**

201 Evolutionary analyses were performed in Bayes Traits (Pagel et al. 2004; Pagel and
202 Meade 2006). We used phylogenetically corrected t-tests (Organ et al. 2007) to explore variation
203 in brain and body size between clades or groups of species. These test for a phylogenetically-
204 corrected association between a binary variable (0 or 1), assigned to the two groups under
205 consideration, and the phenotype of interest. BayesTraits implements Phylogenetic Least Squares
206 to account for phylogenetic non-independence by converting the phylogeny into a variance-
207 covariance matrix, where the diagonal of the matrix gives information on the path length from
208 root to tips (the ‘variance’) and the off-diagonal values of the matrix provide information on the
209 shared evolutionary history of any pair of species (the ‘covariance’) (Pagel 1997, 1999).

210 Ancestral state reconstructions were performed following Organ et al. (2007) as
211 implemented in Bayes Traits. Bayes Traits assumes a constant-variance Brownian motion model
212 but adopts a model-building approach to test for deviation from the null-model. The constant-
213 variance random-walk model has one parameter, α , which describes the instantaneous variance
214 of evolution (Pagel 1997). This is the default model where all branch length scaling parameters
215 (λ , κ , δ) equal one (Pagel 1997,). These parameters account for deviation from the null model: λ
216 reveals to what extent the phylogeny predicts the pattern of covariance between species, κ
217 stretches and compresses branch lengths and accounts for stasis in longer branches, and δ scales

218 path lengths and accounts for variation in the importance of temporally early or late change.
219 These parameters were estimated using maximum likelihood, and where a parameter was
220 significantly different from 1, as determined using a likelihood ratio test ($-2(\ln[Lh(null\ model)] -$
221 $\ln[Lh(alternative\ model)])$), the value for that parameter was estimated in the final model (Organ et
222 al. 2007).

223 Using the model with the highest likelihood, one can test if a directional-change random-
224 walk model improves the fit to the data. This is the formal test of whether the trait of interest
225 evolved through time with a directional trend. The directional random-walk model has an
226 additional parameter (β) that captures the directional change using a regression between trait
227 values and the total path length (Pagel 1997, 1999). The harmonic means of the likelihoods of
228 the directional and non-directional random walk models are compared with Bayes Factors (Kass
229 and Raftery 1995; Gilks et al. 1996) to determine which model fits the data best. The Log(Bayes
230 Factor) is computed as:

$$231 \quad -2(\ln[Harmonic\ Mean\ of\ Lh(null\ model)] - \ln[Harmonic\ Mean\ of\ Lh(alternative\ model)]).$$

232 A Log(Bayes Factor) (BF) greater than 2 is taken as positive evidence for a difference between
233 the two models, greater than 5 represents ‘strong’ evidence, and greater than 10 is ‘very strong’
234 evidence (Kass and Raftery 1995). This test for directionality was performed for \log_{10} (brain mass
235 [g]), \log_{10} (body mass [g]) and \log_{10} (EQ) within odontocetes and across all cetaceans. Data from
236 Hippopotamidae were excluded to ensure that models, and inferences drawn from them, are
237 specific to Cetacea. Due to the lack of brain size data for extinct mysticetes, we were unable to
238 test for trends in this clade.

239 Once the final model is obtained it can be used to reconstruct ancestral states. Ancestral
240 state reconstructions were performed for \log_{10} (brain mass [g]) and \log_{10} (body mass [g]).

241 Ancestral states for EQ were calculated from these values. In order to estimate the ancestral state
242 of the last common ancestor of Cetacea and Hippopotamidae, data for two species of
243 Hippopotamidae were included as outgroups (Weston and Lister 2009). In the Supplementary
244 Online Material we provide a detailed comparison between ancestral state reconstructions for
245 key nodes and evidence from the fossil record.

246 Changes in brain mass, body mass and EQ along each branch were calculated by taking
247 the difference between values at consecutive nodes. Rates of evolution for particular branches
248 were calculated by dividing these changes by branch lengths (Gittleman et al. 1996; Organ et al.
249 2007; Montgomery et al. 2010). This approach has the caveat that it involves many estimated
250 values ($2n-1$) from only n data points and risks artificially inflating the degrees of freedom and
251 pseudoreplication (Pagel 1994). However, we use it here as a descriptive tool. Notable branch-
252 specific changes, discussed below as ‘major decreases/increases’, are branches with changes in
253 $\log_{10}(\text{phenotype})$ more than one standard deviation from the mean change across the group of
254 interest.

255 Comparisons between the average rates of evolution in primates and cetaceans were
256 performed using Welch’s t-test as the variance between the two groups was significantly
257 different (F-test). Data for primates (Montgomery et al. 2010) were converted to match the units
258 of the current paper (from $(\Delta\log(\text{mass}[\text{mg}])/\text{branch length}[\text{millions of years}])$ to
259 $(\Delta\log(\text{mass}[\text{g}])/\text{branch length}[\text{millions of years}])$ for brain mass, and from residual brain size to
260 EQ for relative brain mass). Only branches present in the extant species tree for each clade were
261 considered as Montgomery et al. (2010) assumed fossil lineages formed polytomies with nodes
262 in the extant species tree.

263 Results of the Bayes Traits analyses were obtained using Markov chain Monte Carlo

264 (MCMC) runs with 3.5 million generations, a conservative burn-in of 500,000 generations, and
265 sampling every 100 generations. These settings were sufficient to achieve chain convergence
266 with acceptable range of data deviation values for all nodes except the basal node for which the
267 MCMC chain was run for 7.5 million generations with a burn-in of 3 million generations. All
268 analyses were performed using the default setting of uniform priors (prior range: -100 to +100).
269 Rate deviation was adjusted to obtain an acceptance of the proposed model parameters (above)
270 between 20% and 40%, and during ancestral state reconstructions the data deviation was adjusted
271 to obtain an acceptance rate for each node's estimate between 20-40%. This is the recommended
272 range to ensure that the likelihood surface is efficiently explored (Organ et al. 2007). For full
273 details of rate parameter estimates see Supplementary Online Material (Table S2).

274

275

276 **Results**

277 **Variation in brain and body size among groups**

278 Phylogenetically corrected t-tests (Organ et al. 2007) were used to analyse variation in
279 brain and body size between major groups of cetaceans. Extant mysticetes have significantly
280 larger body masses than extant odontocetes ($t_{40} = 2.079$, $p = 0.044$) but do not have significantly
281 larger brain masses ($t_{40} = 1.000$, $p = 0.323$). This results in mysticetes having significantly lower
282 EQs ($t_{40} = 2.225$, $p = 0.032$). The highest EQs in cetaceans are observed among the
283 Delphinoidea, especially among delphinid dolphins (Marino et al. 2004), however, there are no
284 significant differences between the Delphinoidea and other extant odontocetes for body mass (t_{34}
285 $= 0.107$, $p = 0.916$), brain mass ($t_{34} = 0.669$, $p = 0.508$) or EQ ($t_{34} = 1.227$, $p = 0.228$), or

286 between Delphinidae and other extant odontocetes for body mass ($t_{34} = 0.129$, $p = 0.898$), brain
287 mass ($t_{34} = 0.561$, $p = 0.578$) or EQ ($t_{34} = 1.102$, $p = 0.278$).

288 Extinct odontocetes have significantly smaller body masses ($t_{47} = 2.762$, $p = 0.008$) and
289 brain masses ($t_{47} = 2.147$, $p = 0.037$) than the extant odontocetes in the dataset, but they do not
290 significantly differ in EQ ($t_{47} = 1.812$, $p = 0.076$). Extant odontocetes do not have significantly
291 different body masses ($t_{41} = 0.088$, $p = 0.930$), brain masses ($t_{41} = 1.064$, $p = 0.294$), or EQs (t_{41}
292 $= 1.674$, $p = 0.102$), than stem Cetacea. In contrast, when extant mysticetes are compared to
293 these early stem cetaceans there are no significant differences for brain mass ($t_{11} = 1.527$, $p =$
294 0.155) or EQ ($t_{11} = 1.359$, $p = 0.201$) but extant mysticetes do have significantly larger body
295 masses ($t_{11} = 2.464$, $p = 0.031$).

296

297 **Macroevolutionary trends in brain and body size**

298 The dataset includes fossil taxa that sample a long duration of evolutionary time (Figure
299 2a) providing a good basis for testing macroevolutionary trends. There is very strong evidence
300 that the directional model fits both body mass (BF = 10.208) and brain mass (BF = 10.167) better
301 than the non-directional model, suggesting both traits typically increased through time across
302 cetaceans (Figure 2b, Table 1). However, there is no support for a directional model of evolution
303 when applied to EQ (BF = -0.589). Within odontocetes the same pattern emerges. There is good
304 support for an evolutionary trend to both body mass (BF = 5.779) and brain mass (BF = 5.336)
305 but not for EQ (BF = -1.106). These results are robust to exclusion of extinct taxa with key
306 phylogenetic positions (Supplementary Online Material). Based on branch-specific estimates of
307 phenotypic change, body mass increased across 78% of evolutionary time (total time across all
308 branches of the phylogeny), brain mass increased across 74% of evolutionary time, but EQ

309 increased across only 34% of evolutionary time. Because of these trends, the inclusion of fossil
310 data substantially affects estimates of ancestral states compared to analysis of extant species
311 alone (Supplementary Online Material, Figure S1).

312

313 **Origins and diversification of major groups**

314 **i) Evolution of stem Cetacea and the origins of crown Cetacea**

315 The estimated body and brain masses for the last common ancestor of Cetacea and
316 Hippopotamidae (node 1, Figure 1) are 43.478kg and 71.457g respectively, giving an EQ of
317 0.465. These estimates have a narrow range (Figure S2, Table S3) and should be viewed with
318 some caution given that no close extinct relatives of hippos were included in our study. The most
319 basal node in Cetacea included in our phylogeny (node 2) was probably predominantly aquatic
320 but, like *Dalanistes* and *Rodhocetus* (Gingerich, 2003), capable of some terrestrial locomotion. It
321 is estimated to have had a body mass of 541.330kg (95% CIs: 539.181-543.487kg), within the
322 range of extant odontocetes and representing over a ten-fold increase in body mass relative to the
323 common ancestor of hippos and whales (Figure 3a). Brain mass is estimated to be 308.738g
324 (95% CIs: 308.074-309.404g), a 4.32-fold increase, giving an EQ of 0.371 (Figure 3 a, b). The
325 brain mass is towards the lower end of the range observed within extant odontocetes. The EQ is
326 below the range seen in extant odontocetes but within that of extant mysticetes.

327 Within stem Cetacea, body and brain mass increased in parallel along the lineages
328 leading to crown Cetacea and *Basilosaurus* (Table S4a). The largest shifts during this early
329 period of cetacean evolution are observed between nodes 3 and 4 when body mass is estimated to
330 have increased from 615.262kg (95% CIs: 613.507-617.022kg) to 1,275.073kg (95% CIs:
331 1,268.686-1,281.492kg). A similar increase in brain mass is observed between the same nodes,

332 resulting in a modest increase in EQ (0.356 to 0.464). Large increases in body mass are observed
333 at the origin of *Basilosaurus* (+41%) and in both terminal *Basilosaurus* branches (*B. isis*:
334 +108%, *B. cetoides*: +87%). This is accompanied by increases in brain mass such that EQ
335 remains relatively stable. In contrast, body mass is estimated to have decreased from
336 1,275.073kg (node 4) to 166.571kg (node, 9 [95% CIs: 165.774-167.371kg]) after the split
337 between *Basilosaurus* and the lineage leading to crown Cetacea. This is an overall decrease of
338 87%. Brain mass also decreased, but to a lesser extent, from 685.473g (95% CIs: 683.449-
339 687.391g) to 522.982g (95% CIs: 521.607-524.360g). This results in a major increase in EQ
340 from 0.464 to 1.383, well within the range of modern odontocetes. The increase mostly occurred
341 after the divergence of *Saghacetus* (nodes 8-9).

342

343 **ii) Mysticeti**

344 The last common ancestor of crown mysticetes (node 10) is estimated to have had a body
345 mass of 2800.881kg (95% CIs: 2770.297-2831.803kg), a brain mass of 1229.946g (95% CIs:
346 1222.536-1237.402g) and an EQ of 0.702 (95% CIs: 0.694-0.710) (Figure 3). The origin of
347 extant mysticetes from the last common ancestor of crown Cetacea (between nodes 9-10) is
348 therefore accompanied by a doubling of brain mass but more notably a 16-fold increase in body
349 mass resulting in a decreased EQ. Continued body mass expansion is observed throughout
350 mysticetes (Figure 4a, Table S4b). No branch is estimated to have experienced a decrease in
351 brain or body mass whereas 7 of 11 branches show decreases in EQ. Hence, although we were
352 unable to quantitatively test for directionality due to the lack of brain mass estimates for fossil
353 mysticetes, our ancestral state reconstructions suggest that mysticete brain and body mass
354 evolved with directional trends to increase; but EQ did not. The largest increases are observed on

355 the terminal *Balaena mysticetus* branch, which saw a 32-fold increase in body mass but only a
356 2.2-fold increase in brain mass, and the stem lineage leading to the last common ancestor of
357 Balaenopteridae and Eschrichtiidae (nodes 10-11), which saw a 6-fold increase in body mass and
358 a 2.7-fold increase in brain mass. These two branches, the stem mysticete branch, and the
359 terminal *Balaenoptera musculus* branch show the highest rates of body mass evolution across
360 cetaceans.

361

362 **iii) Odontoceti**

363 In contrast with mysticetes, the early stages of odontocete evolution are characterized by
364 decreases in body mass. The ancestral odontocete (node 15) had an estimated body mass of
365 130.472kg (95% CIs: 129.927-131.022kg), a decrease in body mass of 22% from the ancestral
366 crown cetacean (node 9). Brain mass is estimated to have been 539.533g (95% CIs: 538.282-
367 549.787g), within the 95% confidence intervals for the estimate at node 9. This resulted in an
368 increase in EQ to 1.680 (95% CIs: 1.674-1.687). All three trait estimates are within the range of
369 crown odontocetes (Figure 3). The reduction in body mass was continuous, with all internal
370 branches estimated to have experienced decreases in body mass between the ancestral crown
371 cetacean (node 9) and the ancestral crown odontocete (node 22), where body mass is 86.471kg
372 (95% CIs: 85.967-86.978kg). During this period, brain mass remained relatively constant,
373 between 520-540g, and as a result, EQ climbed steadily from 1.383 to 2.146 (node 22; 95% CIs:
374 2.135-2.157). This pattern of decreasing body mass continued on branches leading to the last
375 common ancestors of Synrhina (nodes 22-27), Delphinida + Ziphiidae (nodes 27-31) and
376 Delphinoidea (nodes 31-35). In each case the decrease in brain mass is limited such that EQ
377 increased. We examined whether this pattern could be due to extant mysticetes inflating the body

378 mass estimate for the ancestral crown cetacean, but even when all mysticetes are excluded from
379 the analysis the same pattern is observed (Supplementary Online Material).

380 The basal branch of Odontoceti (nodes 9-15) shows a large proportional increase in EQ,
381 from 1.383 (95% CIs: 1.377-1.389) to 1.680 (95% CIs: 1.674-1.687). This is partly due to a
382 decrease in body mass but also due to a slight increase in brain mass. Some decreases in body
383 mass also occur within the diversification of crown odontocetes, for example, a 41% decrease at
384 the origin of the Kogiidae (dwarf and pygmy sperm whales; nodes 24-26), and a 48% decrease
385 in *Sotalia fluviatilis*, the tucuxi river dolphin.

386 However, body mass increased across the majority (72%) of evolutionary time during the
387 diversification of the Odontoceti. This compares to 67% for brain mass and just 34% for EQ.
388 Within Odontoceti the largest increases in body mass are observed on branches leading to
389 *Physeter macrocephalus* (107-fold increase from the last common ancestor of Physeteroidea,
390 Figure 4b) and *Orcinus orca* (20-fold increase). During the descent of *P. macrocephalus*, brain
391 mass increased by only 6.5 fold, leading to a decrease in EQ from 2.022 (95% CIs: 2.009-
392 2.035g) to 0.575. This is the second largest EQ decrease across cetaceans. *O. orca* shows a 5.3-
393 fold increase in brain mass, which together with its large body size also leads to a decrease in EQ
394 from 3.867 (95% CIs: 3.851-3.883) to 2.764. Branches with the highest rates of body and brain
395 mass evolution are mostly within the Delphinidae. High rates of increase in both traits are
396 estimated on the terminal *Tursiops truncatus* (Figure 4c) and *Orcinus orca* branches, along the
397 internal branch leading to the last common ancestor of *Tursiops*, *Stenella clymene* and *S.*
398 *coeruleoalba* (nodes 59-61) and the branch leading to the *Pseudorca/Globicephala* clade (nodes
399 54-55; nodes 55-56).

400 Large proportional increases in EQ are observed on the stem lineage to crown Cetacea
401 (nodes 1-9: 0.454 to 1.383), on the stem to crown Odontoceti (nodes 9-22: 1.383 to 2.146) and
402 on the terminal branches leading to *Lagenorhynchus albirostris* and *Globicephala*
403 *macrorhynchus*, both of which show very little change in brain mass. Indeed, body mass
404 decreased on 5/6 branches identified with major increases in EQ. Similarly, the largest decreases
405 in EQ are associated with large increases in body mass. Only *Platanista gangetica* is estimated to
406 have experienced a decrease in brain mass whilst the terminal *Orycterocetus crocodilinus* branch
407 shows large increases in both EQ and brain mass. High rates of EQ increase are observed on the
408 terminal *Delphinus delphis* and *Tursiops truncatus* branches. High rates of EQ decrease are
409 observed on the branch leading to *Stenella clymene* and *S. coeruleoalba*, and terminal *S. clymene*
410 and *Globicephala melas* branches (but note the *G. macrorhynchus* body mass estimate lies
411 towards the lower end of the adult range and may be a sub-adult which could inflate EQ).

412

413 **Brain:body allometry**

414 Changes in brain and body mass are significantly correlated ($t_{81} = 14.670$, $p < 0.001$,
415 Figure S3). We tested whether the discrepancy in EQ values between Mysticeti and Odontoceti
416 reflects a divergence in the allometric relationship between brain and body mass. A similar test
417 was performed between Delphinidae and other Odontoceti, as an allometric shift in delphinids
418 has been suggested (Marino et al. 2004). Based on the available data, the allometric relationship
419 between brain and body mass differs significantly between extant odontocetes and mysticetes (t_{40}
420 $= 2.146$, $p = 0.038$). Within Odontoceti, we find no evidence for an allometric shift in
421 Delphinidae ($t_{34} = 1.286$, $p = 0.207$), or between extant and extinct odontocetes ($t_{45} = 1.681$, $p =$
422 0.100). Hence, within Odontoceti there is no evidence for shifts in the allometric relationship.

423 However, odontocetes do differ from stem cetaceans ($t_{54} = 4.291$, $p < 0.001$). In contrast,
424 brain:body allometry does not differ significantly between extant mysticetes and stem Cetacea
425 ($t_{11} = 1.968$, $p = 0.075$). This suggests that brain:body allometry shifted at least once during the
426 diversification of cetaceans in Odontoceti.

427

428 **Convergent evolution of large brains: a comparison between primates and cetaceans**

429 In contrast to cetaceans, primate evolution has been characterised by a directional trend in
430 brain mass, but not body mass, resulting in a strong trend towards increasing relative brain size
431 (Montgomery et al. 2010). The average rate of body mass evolution is significantly higher in
432 cetaceans than primates ($t_{97} = 2.152$, $p = 0.034$) but the rate of brain mass evolution is
433 significantly higher in primates ($t_{116} = 4.18$, $p < 0.001$). The average rate of change in EQ does
434 not differ between the two orders ($t_{139} = 1.196$, $p = 0.217$). The frequency of decreases in brain
435 mass and EQ is much greater in cetaceans, whereas the frequency of body mass decreases is
436 greater in primates. Across both groups the branches with the highest rates of body mass increase
437 are dominated by cetaceans. In contrast, when ranked by rate of proportional increase in brain
438 mass, only 8 of the 38 branches in the top 25% are cetacean lineages. When ranked for the rate
439 of increase in EQ, 23 of 38 branches in the top 25% are cetacean lineages. The rate of brain mass
440 evolution along the terminal *Homo* branch is exceeded by 5 branches; one cetacean and 4
441 primate lineages. The rate of increase in EQ along the terminal *Homo* lineage is exceeded by 3
442 primate and 4 cetacean branches; with the terminal *T. truncatus* branch having the highest rate.
443 Cetaceans dominate the highest rates of decreases for EQ, brain and body mass. To illustrate the
444 distribution of brain and body sizes through time we plotted data for extinct species and ancestral
445 state estimates for nodes within the extant species tree against time for both primates and

446 cetaceans. It is clear from this that cetaceans reached high levels of encephalization long before
447 primates, and primates only exceeded cetaceans with the emergence of *Homo* (Figure 5).
448 Likewise, the increased variation in cetacean EQ through time is contrasted with the directional
449 progression inferred in primates.

450

451 **Discussion**

452 **Reconstructing the past: effects of incorporating fossils**

453 Understanding the evolutionary processes which shaped past diversity is necessary to
454 understand the origin of extant biodiversity. However, this is challenging using only data from
455 extant taxa. If the evolution of a trait was directionally biased, or characterized by heterogeneous
456 evolutionary rates, reconstructions of past events may be unreliable using extant taxa alone
457 (Oakley and Cunningham, 2000). Including data from extinct taxa may improve such analyses
458 (Finarelli and Flynn 2006; Montgomery et al. 2010; Slater et al. 2012). Although the fossil
459 record is incomplete, particularly for traits such as brain size that require well preserved
460 specimens, a recent simulation study suggests that the inclusion of even a small proportion of
461 extinct diversity can improve our ability to select the best-fitting model of evolution and obtain
462 improved reconstruction of a trait's evolutionary history (Slater et al. 2012). In the present
463 analysis, comparisons of the results obtained with/without fossils and including/excluding taxa in
464 key phylogenetic positions (Supplementary Online Material) suggest that a major factor affecting
465 ancestral state reconstructions in Cetacea is accommodation of directional biases in trait
466 evolution. Hence, in addition to more fully capturing the biological diversity of a group, the
467 inclusion of fossils will improve ancestral state reconstructions if the strength of the directional
468 bias can be reliably estimated from the sample of extinct taxa in the analysis

469 Of course this relies on the dataset being a phylogenetically un-biased sample of past and
470 present diversity (Ackerly, 2000; Freckleton, 2009). With fossil taxa it is difficult to ensure that
471 this is the case, as typically only a small proportion are preserved well enough to measure the
472 phenotype of interest, and new discoveries may reveal previously unappreciated levels of
473 diversity. Although we cannot rule this out, for the present study, we know of no reason to
474 expect a bias in the extinct taxa sampled, either due to preservation and discovery of specimens
475 or data collection. The most likely bias is against early mysticetes, which are not represented in
476 the dataset and could, in theory, result in lower mass estimates for the last common ancestor of
477 extant cetaceans. However, removal of all extant mysticetes and other selected taxa close to these
478 nodes suggest that these estimates are stable.

479

480 **The evolutionary expansion of the cetacean brain: shifts and temporal trends**

481 Increases in both brain and body mass have dominated cetacean evolution. Although EQ
482 has not evolved by a directional, increasing trend in cetaceans, lineage-specific selection
483 pressures presumably resulted in some cetaceans becoming highly encephalized. The initial
484 transition to an obligatory aquatic lifestyle, was not accompanied by a marked increase in
485 relative brain size. This contradicts the hypothesis that an aquatic environment was a key
486 selective pressure that initially drove the increase in cetacean EQ (Marino et al. 2004; Tartarelli
487 and Bisconti 2006) and suggests the shift in brain:body allometry at the origin of crown
488 cetaceans was largely due to changes in body mass development well after the move to aquatic
489 habitats (Manger 2006; Boddy et al. 2012). Despite large differences in EQ, both mysticetes and
490 odontocetes show similar patterns of body and brain mass evolution, with both traits generally
491 increasing through time. Our results suggest the difference between these two sub-orders can

492 largely be explained by a higher rate of body mass evolution in Mysticeti and decreases in body
493 mass at the origin of Odontoceti resulting in an allometric grade-shift between the two sub-
494 orders.

495 The expectation that relative brain size tends to increase through time in mammals
496 (Jerison 1973; Gould 1988) is not met in Cetacea as a whole, or within Odontoceti. Although
497 major changes in EQ occurred independently in several lineages, perhaps indicating lineage
498 specific selection (Marino et al. 2004), the distribution of EQ values across cetaceans is
499 dominated by a major decrease on the stem to crown Mysticeti and a major increase on the stem
500 to crown Odontoceti, in both cases driven by changes in body mass. One hypothesis as to why
501 the brain:body relationship shifted in odontocetes is that selection associated with the evolution
502 of echolocation drove increases in EQ (Marino et al. 2004). The few Oligocene odontocetes
503 whose inner ears have been studied in detail resemble the ears of extant odontocetes (Fleischer
504 1976; Luo and Eastman 1995), suggesting they were specialized for hearing high frequency
505 sounds. However, the phylogenetic positions of these Oligocene taxa are unclear, and it is not
506 known whether the most basal odontocetes, according to the phylogeny of Geisler et al. (2011)
507 (e.g. *Xenorophus*, *Simocetus*, *Archaeodelphis*), had inner ears specialized for high frequency
508 hearing. Although the branch immediately subsequent to the origin of Odontoceti (nodes 9-15)
509 shows a large shift in EQ, shifts in EQ that are caused by major changes in body mass are
510 difficult to interpret. Although we cannot rule out a concurrent remodeling of brain structure, it
511 may be that the stasis in brain size during this period reflects stabilizing selection on brain mass
512 whilst directional selection acted to decrease body mass. If this is the case, the increase in EQ
513 may be neutral with respect to cognition and behavior, although the energetic expense of

514 maintaining a larger relative brain size (Aiello and Wheeler 1995) would suggest that the shift is
515 unlikely to be neutral with respect to overall fitness.

516 Similarly, whether or not the decrease in mysticete EQ suggests anything about their
517 cognitive or behavioral flexibility is unclear as the reduction can largely be explained by huge
518 increases in body mass. Data on mysticete behavior are much more limited than for odontocetes.
519 The available information suggests, however, that social communication and structure are often
520 complex in mysticetes, and include long term social bonds, long range communication,
521 cooperative hunting, cultural traditions, and fission-fusion like social behaviour (Simmonds
522 2006; Marino et al. 2007; Whitehead, 2011). These behaviors are observed in some odontocetes
523 and primates, and are considered cognitively demanding (Barrett et al. 2003; Simmonds 2006).

524 Mysticete body mass is potentially linked to the ability to ingest and process large
525 aggregations of prey (Fitzgerald 2006; Demere et al. 2008). Although the low EQs of mysticetes
526 have previously been attributed to differences in blubber content, which may deflate EQ in large
527 whales, this appears to have a minimal effect (Tartarelli and Bisconti 2006). An additional factor
528 may be increased muscle or bone growth. In primates there is some evidence that EQ is
529 negatively correlated with relative amounts of muscle mass (Muchlinski et al. 2012). This has
530 been attributed to potential energetic trade-offs between brain and muscle tissue (Leonard et al.
531 2007; Muchlinski et al. 2012) but a more parsimonious answer is simply that selection for greater
532 muscle mass acted on postnatal growth and therefore deflated EQ as brain growth ceases earlier
533 in development. Hence shifts in mysticete development and life history could alter EQ by
534 shaping body mass evolution independently of overall brain mass. Although data are limited,
535 mysticetes do appear to have divergent developmental trajectories compared to odontocetes, with
536 extremely high rates of both pre- and post-natal body growth (Frazer and Hugget 1973). To fully

537 interpret changes in EQ caused by changes in either body mass or brain mass a better
538 understanding of the mechanisms and selection pressures causing brain:body allometry is
539 required. The allometric relationship between brain and body size has long been interpreted as
540 evidence of functional or developmental constraints (Jerison 1973), but what these constraints
541 are, whether all regions of the brain are affected equally, and how these traits are linked
542 developmentally is poorly understood (Striedter 2005). Recent studies point towards brain and
543 body mass having independent genetic bases and different aspects of brain development evolving
544 independently (Barton and Capellini, 2011; Montgomery et al. 2011; Hager et al. 2012),
545 suggesting selection can act on brain size and structure, or body mass, without causing correlated
546 shifts in the other.

547 Other neuroanatomical features of mysticete brains, e.g., level of cortical folding and
548 neocortical architecture (Hof and van der Gucht 2007; Oelschläger and Oelschläger 2002) show
549 that they are at least as complex as the brains of some odontocetes. Although mysticete and
550 odontocete brains show some divergent characteristics, particularly in olfactory centers, both
551 clades possess a highly expanded and convoluted cortex and cerebellum suggesting that either
552 major structural changes occurred in parallel in mysticetes and odontocetes or that these changes
553 occurred before the origin of crown Cetacea (Marino et al. 2000; Oelschläger and Oelschläger
554 2002; Marino 2004; Tartarelli and Bisconti 2006; Hof and van der Gucht 2007). Limited data on
555 neuron numbers suggest that, despite large size differences, mysticete and odontocete brains
556 have similar numbers of neurons (Oelschläger and Oelschläger 2002; Eriksen and Pakkenberg
557 2007), both being comparable to the number found in an average chimpanzee brain
558 (Huggenberger 2008). Von Economo neurons, which may be linked to some higher cognitive
559 faculties (Allman et al. 2005; Butti et al. 2013), are found in both mysticetes and odontocetes

560 (Butti et al. 2009) and, as in hominoid primates, show a derived regionally specific distribution
561 (Butti and Hoff, 2010; Butti et al. 2011). Understanding when and why these structural
562 phenotypes evolved would clearly aid our interpretation of cetacean brain evolution.

563

564 **Dissimilarities in the convergent evolution of big brains**

565 Comparing patterns of evolution of cetacean and primate brains provides a potential
566 pathway to uncover shared and divergent evolutionary routes to large brains (Marino 1996, 1998;
567 Tartarelli and Bisconti 2006). We confirm that cetaceans reached high levels of encephalization
568 before primates (Fig. 5; Marino 1998) but our analysis reveals two key differences in brain
569 evolution between primates and cetaceans. First, primates show a strong directional trend for
570 relative brain size to increase through time (Montgomery et al. 2010) whereas cetaceans do not.
571 Second, the discrepancy between the pattern of brain and body mass evolution is much greater in
572 primates than in cetaceans. Whereas cetacean brain:body allometry is predominantly altered
573 during three key periods (the origin of crown cetaceans, the origin of odontocetes and the origin
574 of mysticetes), primate brain and body mass evolved under contrasting selective regimes across
575 longer periods of time resulting in a continuous allometric change and the expansion of relative
576 brain size (Montgomery et al. 2010). Whether this is due to a general decoupling of the genetic
577 basis of brain and body mass evolution in primates, as predicted by quantitative genetics models
578 (Lande 1979), or whether this pattern of brain evolution is unique to primates, remains to be
579 tested. Regardless, the patterns of brain and body size evolution in cetaceans and primates
580 suggest that selection can act on brain mass independently from body mass over discrete periods
581 of major change or in a more continuous and accumulative way over longer periods of time,

582 despite the strong genetic covariance between these traits in mammals (Atchley et al. 1984;
583 Riska and Atchley 1985).

584 Understanding the underlying developmental mechanisms that influence the differences
585 in macroevolutionary trends between primates and cetaceans may ultimately help to identify
586 differences in the constraints and selection pressures acting in these two orders. For example,
587 Lande (1979) postulated that the genetic basis of brain and body mass evolution in primates may
588 have become increasingly decoupled in order to facilitate an evolutionary expansion of the brain
589 without leading to gigantism and antagonistic selection on body mass. In cetaceans, the evolution
590 of large body masses may have been less of a constraint. Physical constraints acting on body
591 mass may differ between cetaceans and terrestrial mammals as cetaceans experience “aquatic
592 weightlessness” (Marino 1998; Huggenberger 2008). It has been argued that this will lead to
593 underestimates of encephalization for many cetaceans when compared to terrestrial mammals
594 (Harvey and Krebs 1990). It is likely, however, that the aquatic lifestyle of cetaceans also results
595 in altered physical constraints on brain size when compared to arboreal or terrestrial mammals
596 such as primates, for which a larger head may result in substantial costs related to locomotion.
597 Similarly, a tradeoff between relative brain size and the amount of adipose depots has recently
598 been demonstrated across mammals (Navarrete *et al.* 2011). This trade-off is postulated to be due
599 to locomotor constraints associated with carrying fat depots conflicting with the need for fat
600 storage as a means of surviving periods of low food availability. Shifts in such constraints in
601 marine habitats likely results in a rebalancing of this trade-off and may contribute to the altered
602 brain:body allometry seen in cetaceans.

603 These, or other, differences in constraints, such as the absence of a limiting bony birth
604 canal in cetaceans (Connor 2007), may explain why the evolution of the cetacean brain

605 proceeded along a unique path (Marino 2004b; Hof et al. 2005). Whilst terrestrial mammals
606 appear to have increased the computational power of their brains by adding new modules to
607 increase structural complexity (Striedter 2005), cetaceans have pursued an alternative route to
608 complexity by multiplying existing structures (Morgane et al. 1990; Marino 2004b;
609 Huggenberger 2008) resulting in a thin but highly folded cortex (Morgane et al. 1990; Marino
610 2004b; Huggenberger 2008). This type of elaboration may have not been available to smaller
611 bodied terrestrial mammals due to mechanical and gravitational constraints favoring more size-
612 efficient schemes of cortical elaboration.

613

614 *Future directions*

615 We suggest comparative data on brain structure and neuron numbers (Stephan et al. 1981;
616 Herculano-Houzel et al. 2007) will be a necessary next-step to provide a fuller interpretation of
617 the significance of brain size differences. A complementary approach may be to investigate the
618 genetic basis of cetacean brain and body mass evolution. Comparative genomics could reveal
619 whether or not the same genes implicated in primate brain evolution have been targeted by
620 selection in cetaceans (McGowen et al. 2011; McGowen et al. 2012; Xu et al., 2012), or may
621 reveal categories of genes which evolved adaptively with functional relevance to cetacean-
622 specific neural phenotypes (McGowen et al. 2012). Combining approaches from neuroanatomy,
623 comparative biology, paleontology and evolutionary genetics will lead to new insights into the
624 origins of behavioral complexity in cetaceans and the convergent evolution of large brains across
625 divergent mammalian orders. Our analysis provides a comparative framework for interpreting
626 future analyses of brain evolution in Cetacea.

627

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633

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871 **Figure Legends**

872

873 **Figure 1:** Phylogeny of extant and extinct cetaceans for which brain and body mass estimates
874 are available. Nodes are labeled and referred to throughout the text. Branches which show an
875 increase (upward pointing arrows) or decrease (downward pointing arrows) more than one
876 standard deviation from the mean change across all branches are labeled for body mass (blue),
877 brain mass (red) and EQ (green). Scale bar for branch lengths (three millions years [MY]) is
878 shown at bottom left. Paintings are by Carl Buell.

879

880 **Figure 2:** Temporal trajectories in brain and body mass: **A)** Scatterplots of \log_{10} i) body mass, ii)
881 brain mass and iii) EQ against time (millions of years ago [MYA]). Green dots: Archaeoceti,
882 blue dots: Odontoceti, brown dots: Mysticeti, red dots: ancestral state estimates for 5 key nodes
883 (1 = ancestor of Cetacea + Hippopotamidae, 2= ancestor of Cetacea, 9 = ancestor of crown
884 Cetacea, 22 = ancestor of crown Odontoceti, 10 = ancestor of crown Mysticeti). **B)** Posterior
885 distribution of likelihoods for non-directional (red/brown) and directional (blue) model of
886 evolution for \log_{10} i) body mass, ii) brain mass and iii) EQ.

887

888 **Figure 3:** Posterior distribution of ancestral state estimates for 5 key nodes (red: node 2, blue:
889 node 9, green: node 10, purple: node 15, yellow: node 22) for \log_{10} **A)** body mass, **B)** brain mass
890 and **C)** EQ. The upper panels show dot histograms indicating the distribution of extant
891 phenotypes for odontocetes (dark grey circles) and mysticetes (light grey squares).

892

893 **Figure 4:** Evolutionary trajectories along selected lineages: Log(body mass[g]) is shown by the
894 blue lines, Log(brain mass[g]) by the red and EQ (not logged) in green. **A)** *Balaena mysticetus*
895 (dashed line) and *Balaenoptera musculus* (solid line) provide examples of enormous body mass
896 increases and a falling EQ in spite of an increase in brain mass, **B)** *Kogia sima* (dashed line) and
897 *Physeter macrocephalus* (solid line) provide examples of a large bodied odontocete evolving a
898 small EQ compared to a smaller bodied relative, **C)** *Tursiops truncatus* exemplifies a species
899 where EQ has increased rapidly and recently due to brain mass expansion and **D)** *Neophocaena*
900 *phocaenoides* provides an example where brain mass is relatively constant over a long period of
901 time but EQ increases mostly due to decreases in body mass. Where two species are represented
902 in the same panel the line is solid until the point at which they diverge then one line is dashed
903 and the other remains solid. Dots represent internal nodes from the extant species tree leading
904 from the last common ancestor of Cetacea and Hippopotamidae to each extant species. MYA =
905 millions of years ago. Paintings are by Carl Buell.

906

907 **Figure 5:** Distribution of log(body mass [g]) (**A**), log(brain mass [g]) (**B**) and log(EQ) (**C**)
908 through time for cetaceans (blue) and primates (red) from their origins to present day (x-axis is
909 millions of years ago [MYA]). Scatterplots include data for extant species, extinct taxa, and
910 ancestral states reconstructed at internal nodes of the tree in Fig. 1. Paintings are by Carl Buell.

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916 **Tables**

917

918 **Table 1: Statistical tests for macroevolutionary trends in brain and body size**

919 **a) All cetaceans**

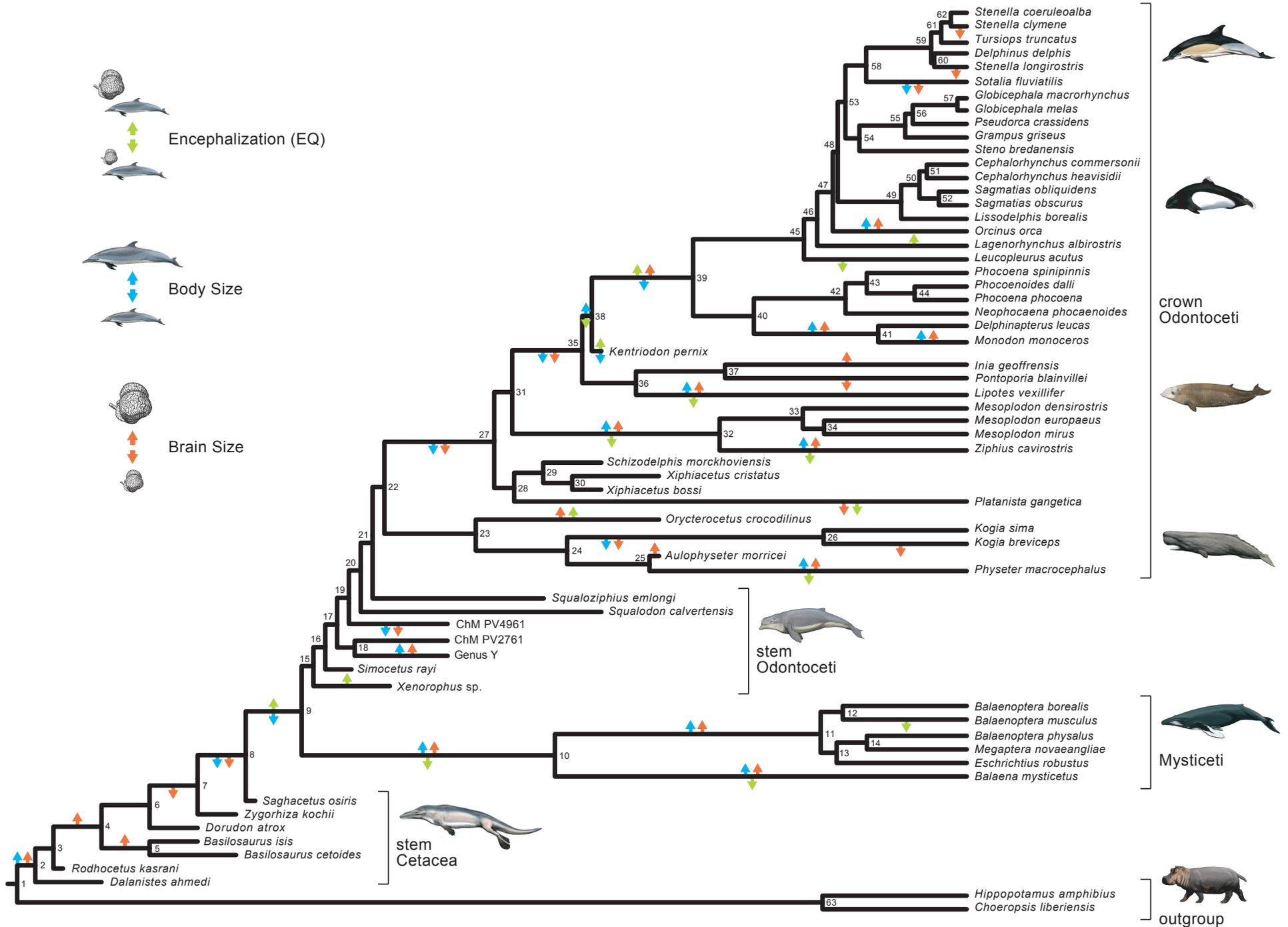
Harmonic mean of Log(likelihoods)	Body mass	Brain mass	Relative brain size (Log[EQ])
Non-directional model	-49.982	-13.474	10.453
Directional model	-44.878	-8.390	10.159
Log(Bayes factor)	10.208	10.167	-0.589

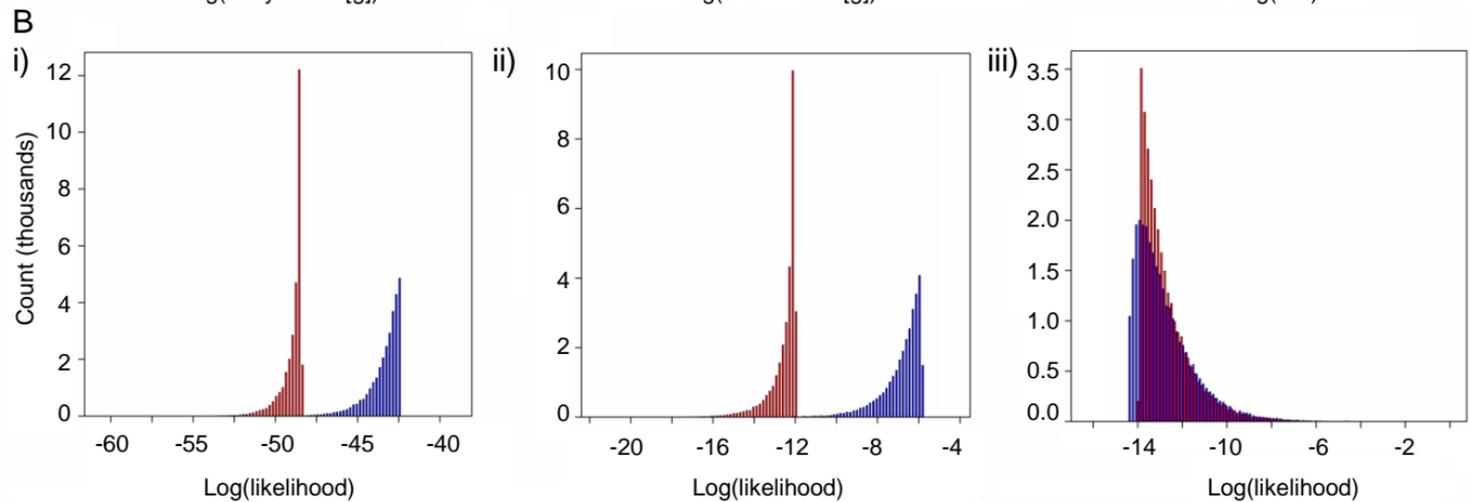
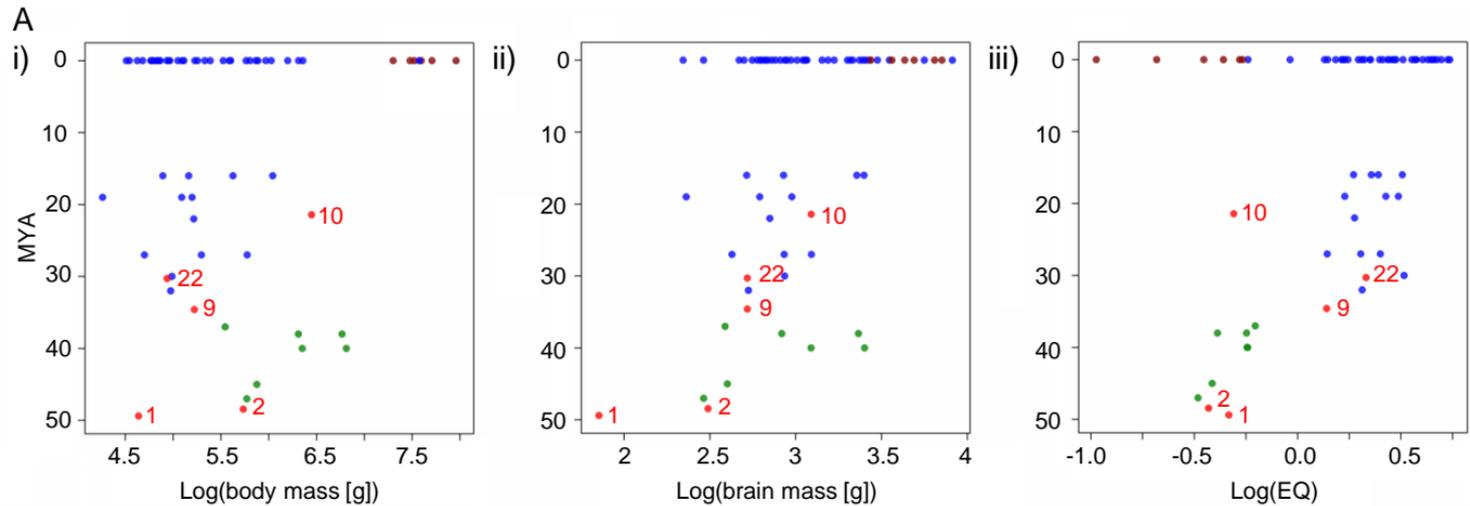
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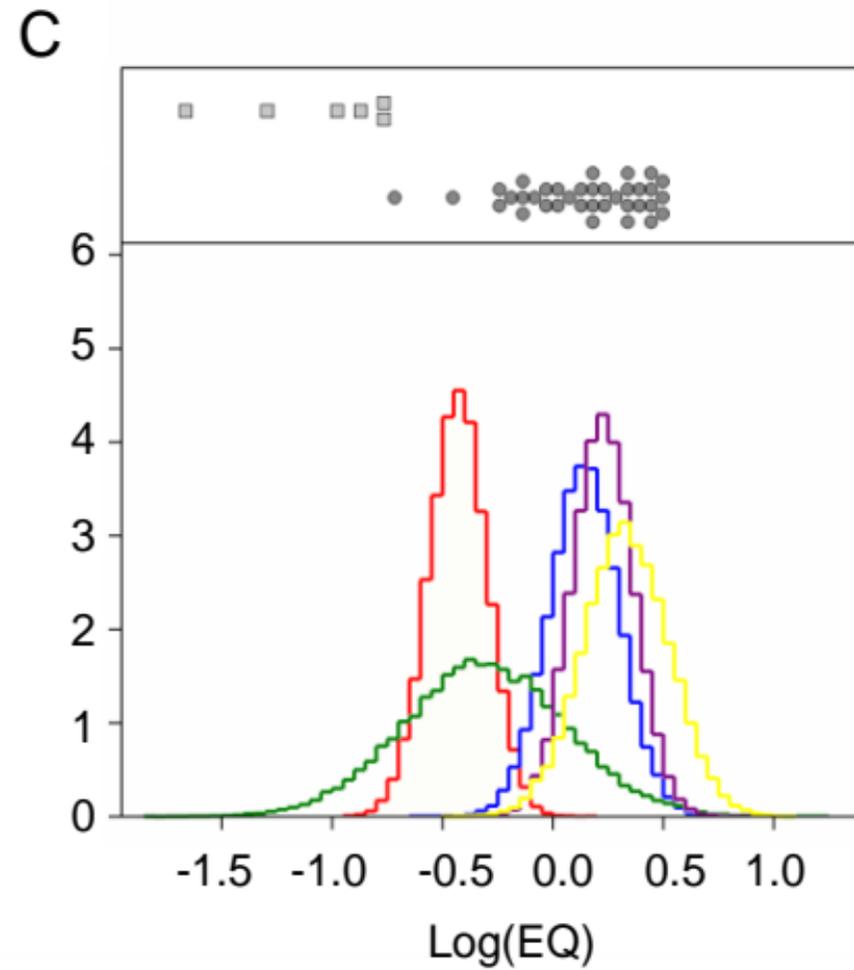
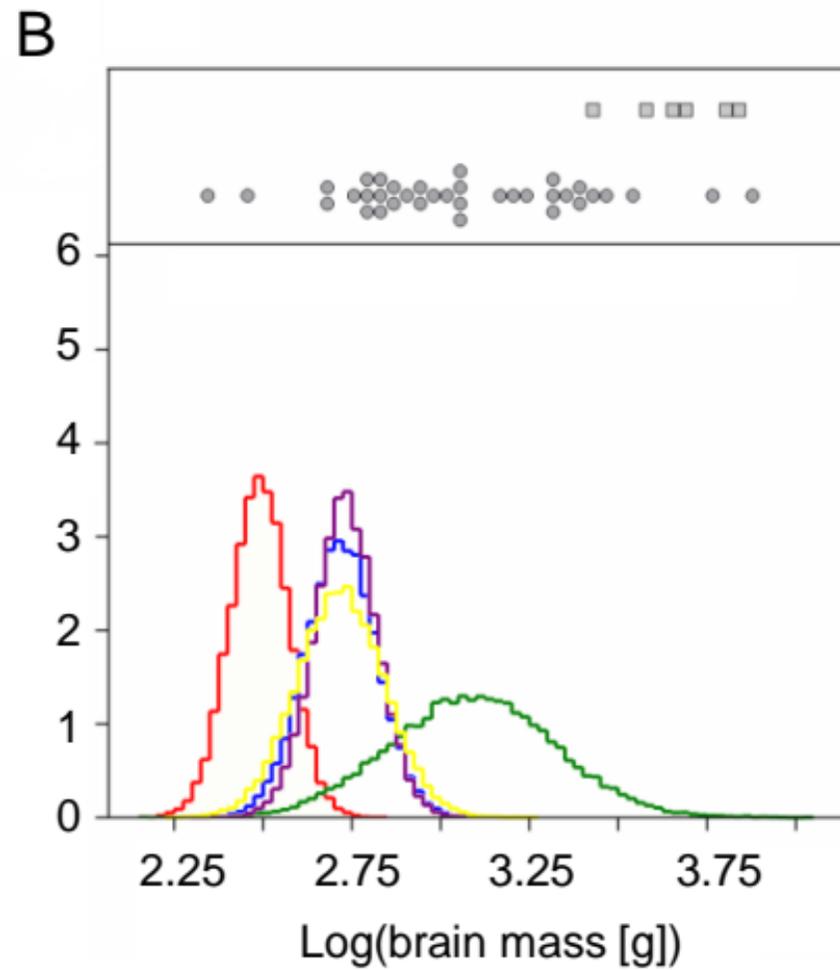
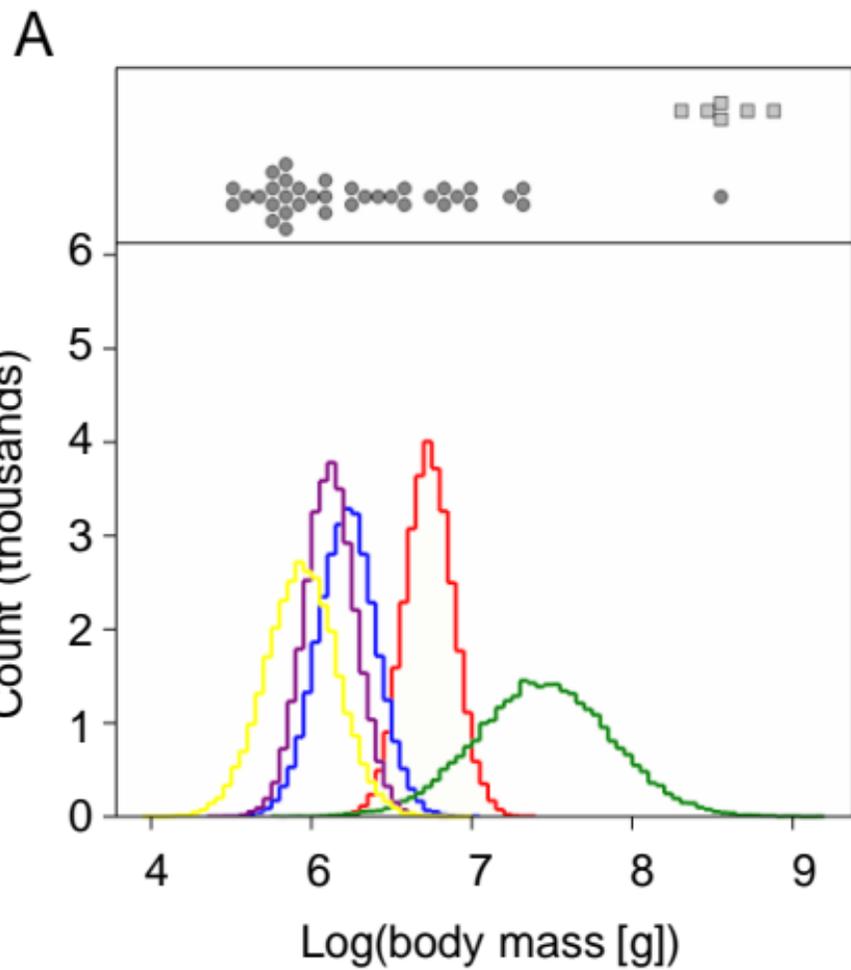
921 **b) Odontocetes only**

Harmonic mean	Body mass	Brain mass	Relative brain size (Log[EQ])
Non-directional model	-36.281	-8.161	9.724
Directional model	-33.391	-5.493	9.171
Log(Bayes factor)	5.779	5.336	-1.106

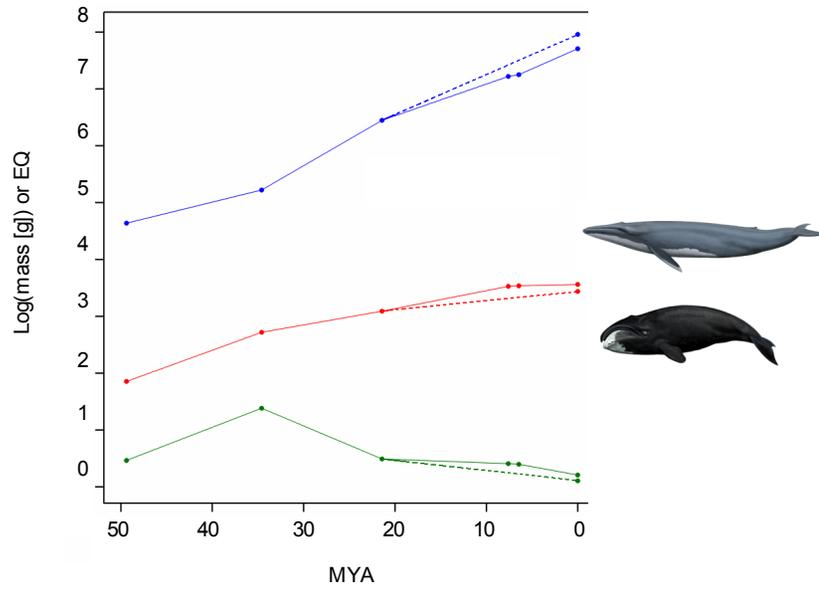
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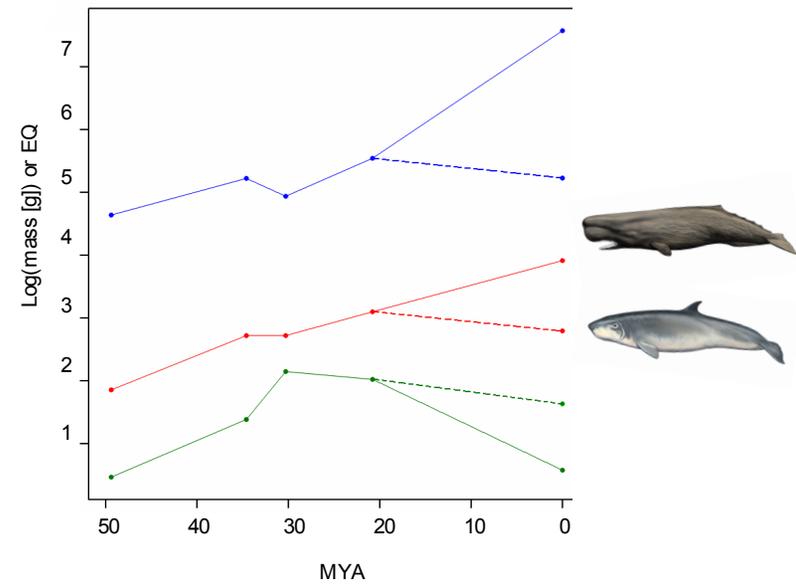




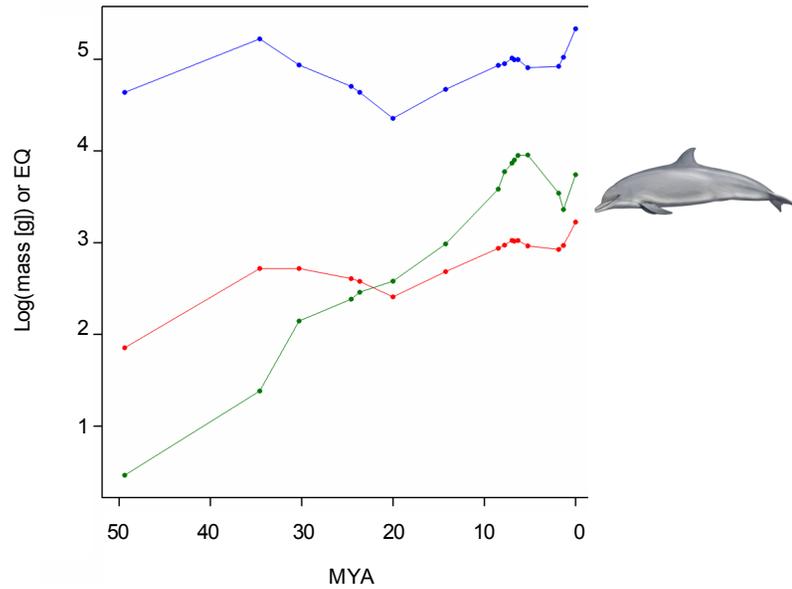
A *Balaena mysticetus*
Balaenoptera musculus



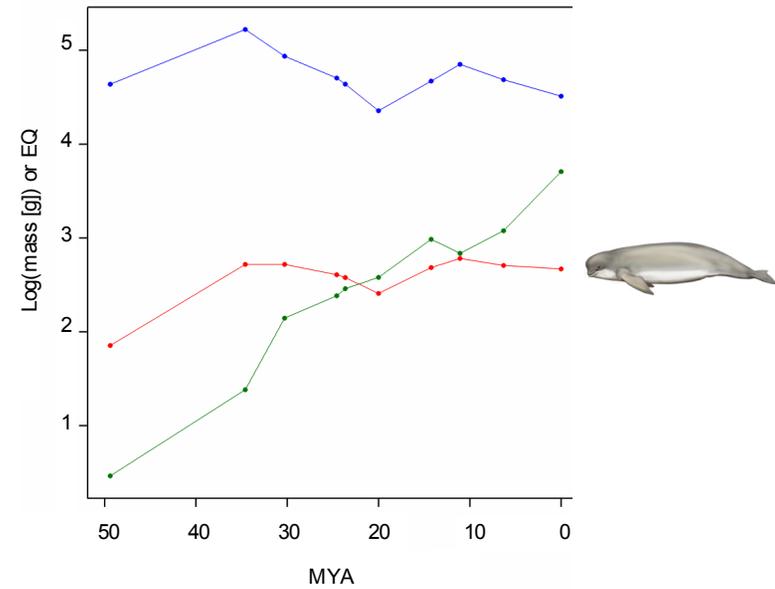
B *Physeter macrocephalus*
Kogia sima

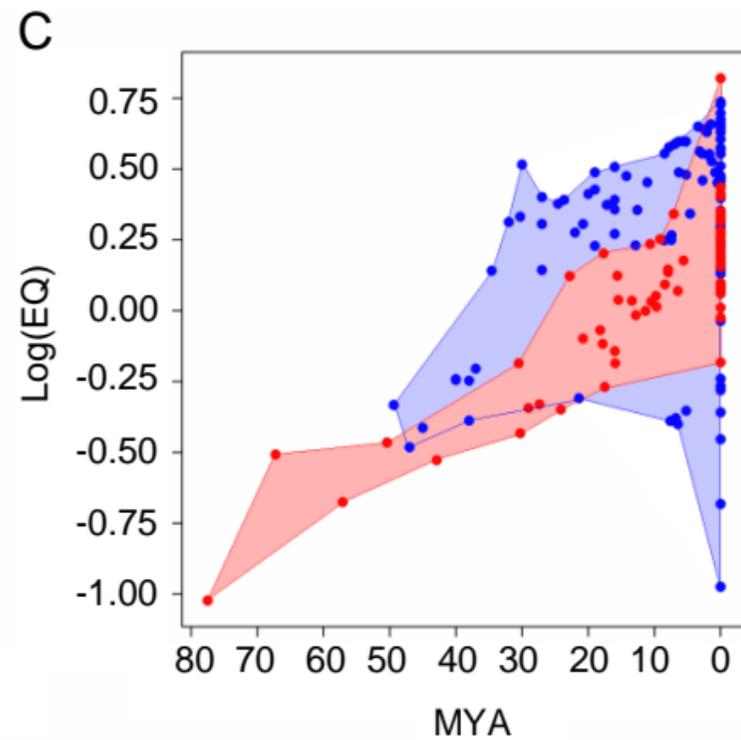
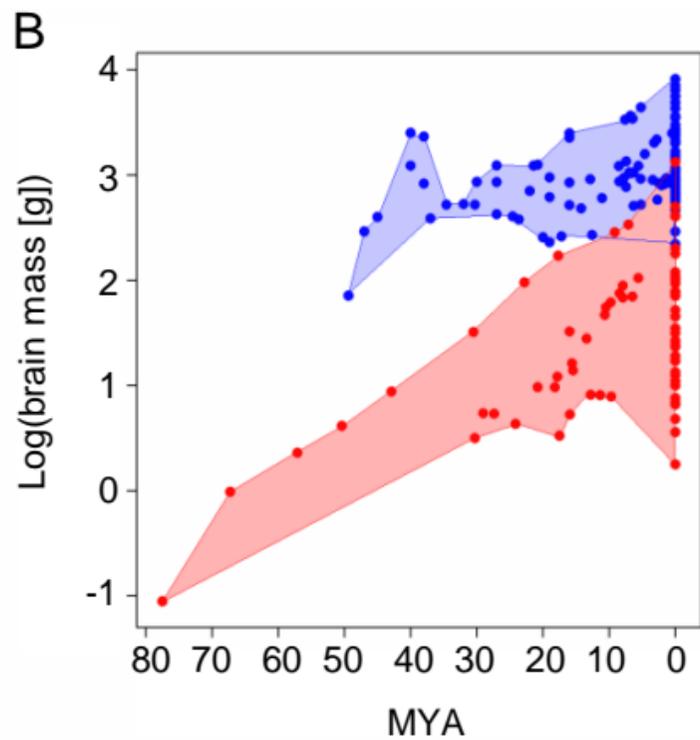
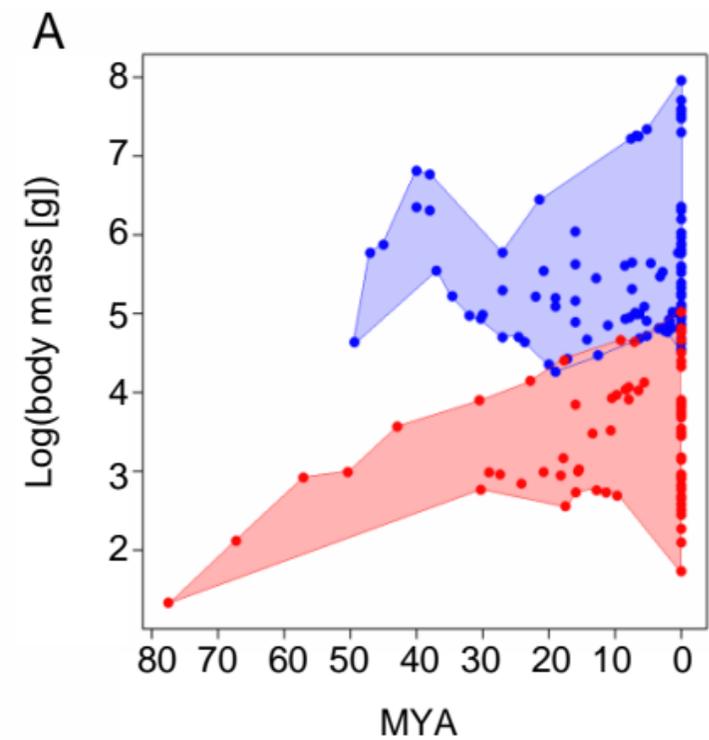


C *Tursiops truncatus*



D *Neophocaena phocaenoides*





The evolutionary history of cetacean brain and body size

Supplementary Information

Stephen H. Montgomery, Jonathan H. Geisler, Michael R. McGowen, Charlotte Fox, Lori Marino, John Gatesy,

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2. Scaling parameters and model building in Bayes Traits
 - Table S2: Scaling parameters
3. Effects of including fossils and accounting for directional trends on ancestral state reconstructions, and alternative measures of relative brain size
 - Figure S1: Scatterplots of ancestral state estimates made with and without fossil data
4. Figure S2: Posterior distribution of ancestral state reconstructions for node 1, the last common ancestor of Cetacea and Hippoptamidae
5. Effects of excluding selected taxa
6. Estimating extinct data to gauge model accuracy
7. Comparison between ancestral state estimates at key nodes and the fossil record
 - Table S8: Measurements and body size estimates for select fossil cetaceans.
8. Figure S3: Regression between changes in cetacean brain and body mass

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- Sheet 5. Table S4b: Changes in phenotypic traits during early odontocete evolution
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- Sheet 7. Table S5: Tests of directionality excluding selected taxa
- Sheet 8. Table S6: Ancestral states of key nodes excluding selected taxa
- Sheet 9. Table S7: Estimating extinct species traits

1. Building the phylogeny

The phylogenetic hypothesis for extant and extinct species is a composite based on previously published molecular (McGowen et al. 2009; McGowen 2011) and morphological trees (Geisler et al. 2011). To construct this phylogenetic framework we began with the time-calibrated phylogeny of McGowen et al. (2009) and adjusted relationships within Delphinidae following McGowen (2011). Molecular dating analysis was then re-run in BEAST v. 1.4 (Drummond et al. 2006) using a log-normal uncorrelated relaxed clock and cytochrome *b* sequences as in McGowen et al. (2009) and the multiple fossil calibration points suggested by Geisler et al. (2011) to retrieve time-calibrated branch lengths for this new configuration. The nodes listed below were given log-normal prior distributions with the following parameters: 1) Cetancodonta (47.0 million years [Ma]. 3.0 mean, 0.5 standard deviation [st. dev.]) based on *Pakicetus inachus*, 2) Crown Cetacea (34.2 Ma, 1.0 mean, 1.0 st. dev.) based on the extinct mysticete *Llanocetus denticrenatus*, 3) Crown Mysticeti (20.0 Ma, 2.0 mean, 0.5 st. dev.) based on *Morenocetus parvus*, 4) Synrhina (20.0 Ma, 2.0 mean, 2.0 st. dev.) based on *Notocetus vanbenedeni*, 5) Delphinida (18.5 Ma, 1.0 mean, 1.0 st. dev.) based on *Kentriodon pernix*, 6) Crown Ziphiidae (13.2 Ma, 1.0 mean, st. dev. 0.5) based on *Archaeoziphius microglenoideus*, and 7) Monodontoidae (7.5 Ma, 2.0 mean, 0.5 st. dev.) based on *Salumiphocoena stocktoni*. We then pruned all taxa without brain/body mass data and this tree was then used as a molecular scaffold on which to position extinct taxa. Several molecular clock analyses of Cetacea have been published recently (McGowen et al., 2009; Steeman et al. 2009; Xiong et al. 2009; Ho and Lanfear 2010; Morin et al. 2010; Slater et al. 2010; Chen et al. 2011; Zhou et al. 2011; Dornburg et al. 2012); the dates obtained here are in-line with the majority of these studies.

The molecular scaffold used by Geisler et al. (2011) is entirely consistent with the molecular tree used in the present study; therefore, figure 6 of that study was used to place the following extinct taxa for which brain and body mass estimates are available: ChM PV2761 (undescribed species), *Simocetus rayi*, ChM PV4961 (undescribed species), *Squalodon calvertensis*, *Squaloziphius emlongi*, *Orycterocetus crocodilinus*, *Kentriodon pernix*, *Xiphiacetus bossi*. Unpublished observations by JHG indicate that a specimen of an undescribed species of *Xenorophus*, ChM PV4266, is conspecific with another specimen, ChM PV4823, which was included in the phylogenetic analysis of Geisler et al. (2011). Brain and body size estimates are available for ChM PV4266 from Marino et al. (2004), so we have placed ChM PV4266 in the

phylogenetic position of ChM PV4823 as determined by Geisler et al. (2011). Whitmore and Sanders (1976) provisionally referred to a new taxon of Oligocene odontocete as “Genus Y” and mentioned that multiple specimens of this taxon existed in the collections of the Charleston Museum. Geisler and Sanders (2003) included one specimen of “Genus Y”, ChM PV2764, in their phylogenetic analysis, and these character codings were also used in the scaffold analyses of Geisler *et al.* (2011). ChM PV2757, for which brain and body estimates are available, appears to be conspecific with ChM PV2764, and it is one of the specimens mentioned by Sanders and Whitmore (1976) as belonging to “Genus Y” (AE Sanders, pers. comm.). Thus as with the undescribed specimens of *Xenorophus*, we have positioned ChM PV2757 based on the phylogenetic position of ChM PV2764 in Geisler *et al.* (2011). In figure 1, the new taxon represented by ChM PV4823 and PV4266 is referred to as *Xenorophus* sp. and the new taxon represented by ChM PV2764 and PV2757 is referred to as "Genus Y." Among the extinct taxa included by Geisler *et al.* (2011) for which body and brain size estimates are unavailable, *Notocetus vanbendeni* and *Agorophius pygmaeus* are particularly important because their inclusion yields older divergence estimates. Thus we incorporated these taxa into our method of estimating branch lengths (see below) even though they cannot be used in our tests of temporal trends in brain and body sizes.

Ten additional extinct cetaceans were added to our phylogeny based on three morphological cladistic analyses: *Dalanistes ahmedi*, *Rodhocetus kasrani*, *Zygorhiza kochii*, *Saghacetus osiris*, *Dorudon atrox*, *Basilosaurus cetoides*, *Basilosaurus isis* (based on Uhen, 2004), *Xiphiacetus cristatus*, *Schizodelphis morckhoviensis* (based on Lambert, 2005), *Aulophyseter morricei* (based on Lambert *et al.*, 2010). In each case, these analyses included an overlap of species with Geisler *et al.* (2011), thus it was fairly straightforward to incorporate the fossils from these studies into our composite phylogeny.

The addition of extinct taxa to the molecular tree created new terminal branches that led to these extinct taxa (i.e., none were considered ancestral), and the connection of these branches subdivided branches in the phylogeny of extant taxa. We have expressed the branch lengths of our tree of extant taxa in millions of years, based on our molecular clock study. Thus the age of each fossil we included constrains one end of its terminal branch. The other end of the terminal branch, which is also the point that subdivides a branch on the tree of extant taxa, is unconstrained. In the absence of a data driven way to subdivide these branches, we strove for

consistency. We attached extinct taxa to the tree of extant taxa so that branches are subdivided into two branches of equal duration. To prevent any one extinct taxon from having an undue influence on our reconstruction of ancestral states, we pushed divergences involving fossils back in time so that terminal branches leading to fossils and any internal branches were ≥ 0.5 Ma.

In three instances (i.e. attachment of *Aulophyseter*, *Saghacetus*, and *Rodhocetus*) equal subdivision of branches led to logical contradictions. For example, on the tree of extant taxa, the terminal branch leading to *Physeter* is 20.79 million years in duration. The extinct physeterid *Aulophyseter* is the sister-group to *Physeter* in our tree, and thus its inclusion will subdivide the terminal branch leading to *Physeter* into two new branches, one internal and the other terminal. Equal subdivision would lead to two branches approximately 10.4 Ma each, however, this estimate is not possible because *Aulophyseter* itself is 16 Ma in age. Thus we set the terminal branch leading to *Aulophyseter* to be 0.5 Ma in duration (the minimum), which sets the split between *Aulophyseter* and *Physeter* at 16.5 Ma. The two “new” branches created by the addition of *Aulophyseter* are thus 16.5 Ma (terminal leading to *Physeter*) and 4.29 Ma (ancestral internal branch leading to *Aulophyseter* and *Physeter*). Similarly, *Saghacetus* and *Rodhocetus* were connected to the tree via 0.5 Ma terminal branches. In each of these cases additional fossils needed to be attached above or below these points in time, and equal subdivision of branches was then applied.

The median value for the age of the emergence of Delphinida is approximately 19 Ma. However, this is younger than the best estimate for the age of *Kentriodon pernix* (19.5 Ma), which has been placed as a basal delphinidan (Geisler et al. 2011). This occurred because *Kentriodon pernix* was used as a calibration point in the molecular clock analysis (see above). To resolve this paradox and to prevent *Kentriodon* from sitting on the ancestral branch leading to extant delphinoids, we arbitrarily set the age of Delphinida to be 1 Ma older, at 20 Ma. Then *Kentriodon pernix* was attached to the tree of extant taxa via a 0.5 Ma long terminal branch, and a 0.5 Ma internal branch separated it from the node of crown Delphinida.

There are three wholly extinct clades in our tree: Eurhinodelphinidae (*Xiphiacetus* + *Schizodelphis*), *Agorophius* + Genus Y + ChM PV2761, and *Basilosaurus* (note that *Agorophius* is not in our final tree, Figure 1, but was referenced for dating splits). Here too we positioned divergences so that branches were equal in length. To do this, first we took the age of the older of the two sister species in the most apical position within each clade. We then subtracted this

age from the age of the node by which this extinct clade attached to the tree of extant taxa. Finally, this value was divided by the number of internodes, plus one for a terminal branch. For example, among our sampled taxa, the genus *Xiphiacetus* forms the most apical clade within Eurhinodelphinidae. Of the two species we sampled (*X. bossi* and *X. cristatus*), *X. bossi* shows up first in the fossil record, at 19 Ma. We estimated that Eurhinodelphinidae diverged from *Platanista* 23.54 Ma, thus the difference between this divergence and the age of *X. bossi* is 4.54 Ma. This value was divided by three to yield three branches 1.51 Ma in length: the terminal branch leading to *Xiphiacetus bossi*, the internal branch leading to the genus *Xiphiacetus*, and the internal branch leading to Eurhinodelphinidae.

2. Scaling parameters and model building in Bayes Traits

Ancestral state reconstruction in Bayes Traits has two steps (Organ et al. 2007). First, a model of evolution is obtained which best fits the data and phylogenetic hypothesis. Second, this model is used to estimate ancestral states. In the first step one estimates three scaling parameters under a constant variance (non-directional) model. These are lambda, reveals to what extent the phylogeny predicts the pattern of covariance between species for a trait (the phylogenetic signal); kappa, stretches and compresses branch lengths and tests for stasis in longer branches; and delta, scales path lengths and tests for adaptive radiations or a greater importance of temporally early change. If these parameters are estimated to be significantly different to one (the default) they are estimated in the final model. Once the best constant variance model has been established it is compared to a directional model where the same scaling parameters are estimated. Scaling parameters were estimated using maximum likelihood. Only lambda differed significantly from one (lambda ML estimate = 0.970) for EQ across all cetaceans (Table S2). Within odontocetes both lambda (ML estimate = 0.904) and kappa (ML estimate = 0.575) were significantly different from one.

These parameter estimates suggest EQ is more phylogenetically labile than body mass or brain mass. In addition the low kappa estimate suggests an element of stasis on longer branches within the odontocetes. In agreement with previous analyses (Slater et al. 2010) we find no evidence for an early burst of body size diversification in cetaceans, as would be indicated by values of delta which are significantly below one.

Comparisons of directional and non-directional models were carried out using a Bayesian MCMC framework incorporating scaling parameters that differed significantly from one (i.e. lambda for EQ). The statistics for these tests are reported in the main text. For the ancestral state reconstructions, two species of Hippopotamidae were added to enable estimates of the last common ancestor of Cetacea and Hippopotamidae. Scaling parameter estimate for the dataset including these species produced nearly identical results, but the Bayes Factors for the non-directional/directional test were reduced slightly for both brain (harmonic mean of non-directional model = -14.585, harmonic mean of directional model = -10.282; Bayes Factor = 8.906) and body mass (harmonic mean of non-directional model = -52.600, harmonic mean of directional model = -48.801; Bayes Factor = 7.598).

Table S2: Scaling parameters for**a) All cetaceans**

Trait	n	Null	Likelihoods			p-value sig. diff. from 1		
			lambda	kappa	delta	lambda	kappa	delta
Brain mass	64	-13.425	-13.426	-12.245	-12.221	0.989	0.124	0.827
Body mass	64	-51.185	-50.627	-51.140	-51.177	0.291	0.766	0.901
Relative brain (EQ)	64	11.249	13.959	12.569	11.267	0.020	0.104	0.850

b) Odontocetes only

Trait	n	Null	Likelihoods			p-value sig. diff. from 1		
			lambda	kappa	delta	lambda	kappa	delta
Brain mass	49	-5.540	-5.516	-5.313	-5.251	0.826	0.500	0.448
Body mass	49	-34.962	-33.948	-34.806	-34.342	0.155	0.577	0.266
Relative brain (logEQ)	49	18.129	25.706	21.469	19.136	<0.001	0.010	0.156

3. Effects of including fossils and accounting for directional trends on ancestral state reconstructions, and alternative measures of relative brain size

a) Fossils and directional trends

As they are optimized to take into account particular features of the mode of evolution of a trait, ancestral state reconstructions in Bayes Traits are expected to produce more reliable ancestral state reconstructions (Pagel, 1997, 1999; Organ et al. 2007, Montgomery et al. 2010). Previous ancestral state reconstructions in cetaceans have employed parsimony or maximum likelihood methods which assume a standard Brownian motion model (Marino et al. 2004; Boddy et al. 2012). These methods are not robust to violations of their assumptions of constant rate of evolution and equal probability of change in either direction and can therefore produce misleading results, especially when evolutionary trends shaped the evolution of the trait of interest (Pagel, 1999; Oakley and Cunningham, 2000; Webster and Purvis, 2002; Pedersen *et al.* 2006). The approach implemented in Bayes Traits accounts for deviation from the Brownian motion model and therefore accommodates alternative, more complex evolutionary patterns which may therefore produce more reliable results (Organ et al. 2007; Montgomery et al. 2010).

To examine the effects of including fossil data we repeated the ancestral state reconstructions excluding all fossils. By doing so it is not possible to test for a directional trend in the data or account for it. Hence, without fossil data all phenotypes are analyzed under a constant-variance non-directional model. Sets of reconstructions for nodes within the extant species tree only (i.e. nodes created by extinct species were not considered) made with and without fossil data were compared using Spearman's rank correlation as in Montgomery et al. (2010). Methods are as detailed in the main text.

The two sets of ancestral state reconstructions are significantly correlated for body mass ($t_{38} = 5.62$, $p < 0.001$ $r_s = 0.674$) and brain mass ($t_{38} = 5.09$, $p < 0.001$ $r_s = 0.637$) but the level of congruence between ancestral state estimates made with and without fossil data is low (Figure S1). For both brain and body mass, excluding fossil data leads to larger estimated ancestral states. Montgomery *et al.* (2010) suggest that differences such as these are likely caused by both the inclusion of fossils, and the consequent estimation of directional trends. The nodes with the largest discrepancy between estimates made with and without fossil data tend to be deep nodes at

the bases of clades with large phenotypic shifts (e.g. node 10 – the ancestral mysticete, nodes 22 and 24 – the ancestral odontocete and the origin of the lineage leading to *Physeter*, node 27 to 31 and node 35 to 39 early nodes in Synrhina). This is particularly the case for brain mass. The largest discrepancy for both phenotypes is node 22, the ancestral odontocete, which, without fossil data is estimated to have a more than tenfold larger body mass (1284.407kg vs. 86.471kg) and brain mass (7870.138g vs. 522.926g) without fossil data.

The discrepancy between EQ estimates derived from the brain and body mass estimates is much lower (Figure S2). Again the two sets of estimates, with and without fossils, are correlated but the correlation coefficient is much higher ($t_{38} = 13.04$, $p < 0.001$ $r_s = 0.904$). The majority of estimates are fairly similar with only a few nodes showing large differences – in particular nodes 10, 22, 24, 27 and 31 – all basal nodes. Without including fossil data the ancestral mysticete (node 10) is estimated to have an EQ of 1.144, with fossils the estimate is 0.491. This is the largest discrepancy. These results suggest that the error introduced by excluding the range of size diversity represented by fossil taxa and failing to account for the directional trends in brain and body mass is limited for shallower nodes in the tree but is quite substantial at deep nodes.

Given the statistical support for directional models for brain and body mass and the expectation that including fossil data should improve ancestral state reconstructions we suggest that our results were improved by accounting for these effects. This is in line with previous studies which have shown evolutionary trends can have a large effect on ancestral state reconstructions (Pagel, 1999; Oakley and Cunningham, 2000; Webster and Purvis, 2001; Pedersen et al. 2006; Montgomery et al. 2010).

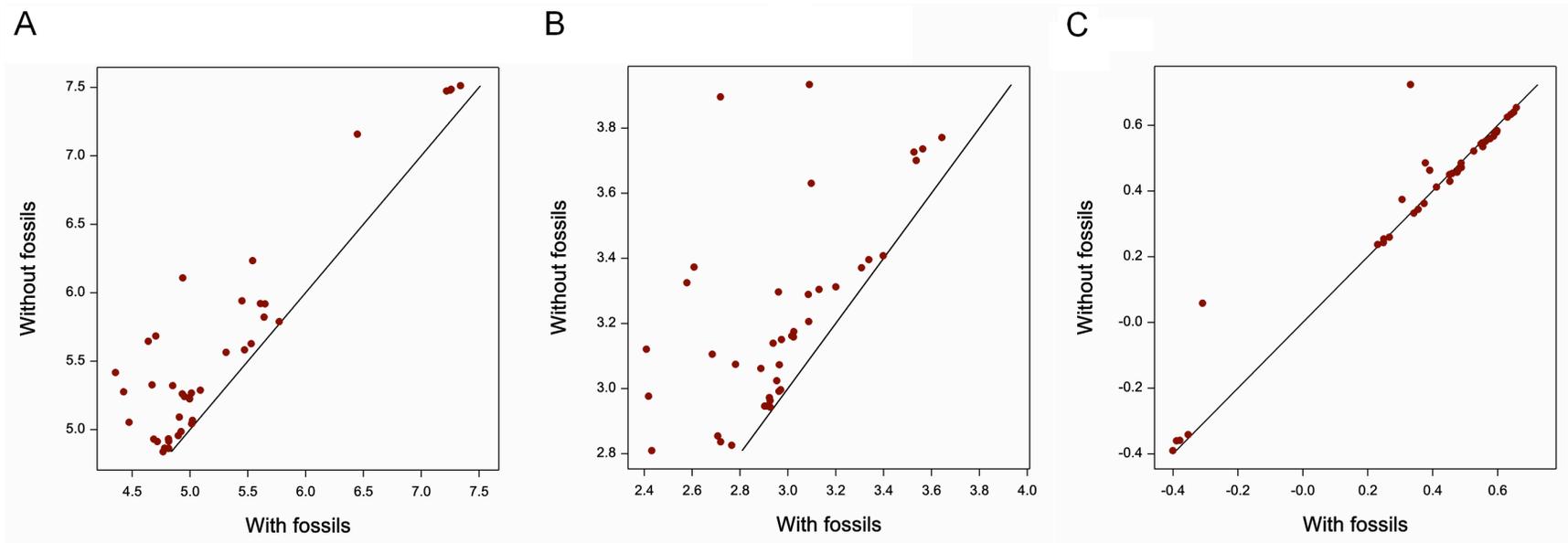
b) Alternative measures of relative brain size

The effects of alternative measures of relative brain size were explored by repeating analyses of relative brain size using residuals from the brain:body regression estimated using Phylogenetic Generalized Least Squares (PGLS) in Bayes Traits (see below) and our dataset of brain and body mass:

$$\text{Residual brain size} = \log_{10}(\text{Brain mass [g]}) - (0.612 * (\log_{10}(\text{Body mass [g]}) - 1.061))$$

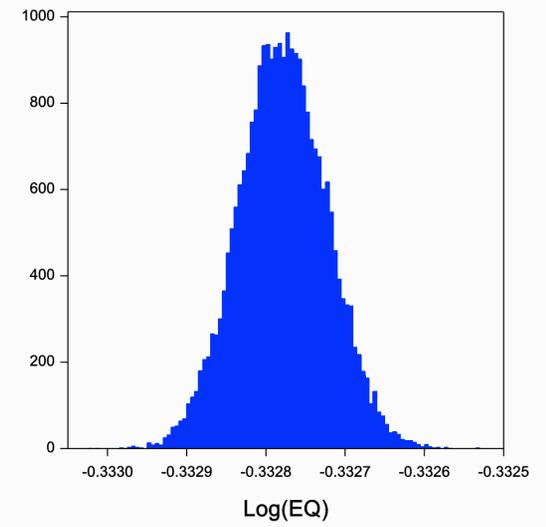
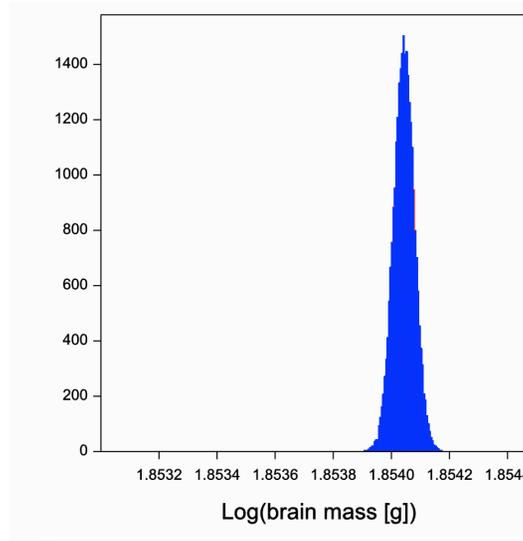
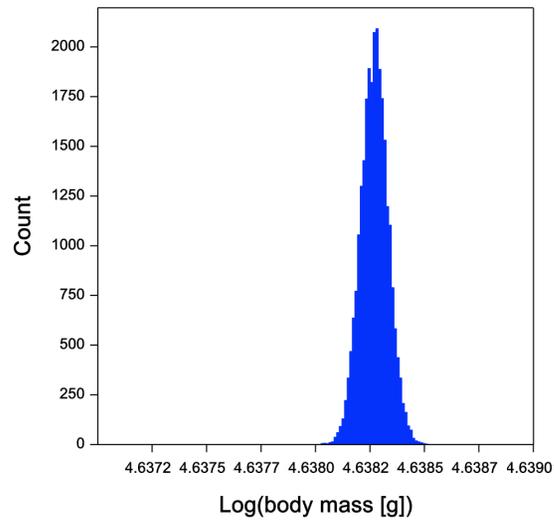
Results between $\log_{10}(\text{EQ}_{67})$ and residual brain mass were strongly associated ($t_{60} = 63.090$, $p < 0.001$, $R^2 = 0.991$), and therefore only results of the analyses using EQ are reported here.

Figure S1: Scatterplots of ancestral state estimates made with and without fossil data* for A) body mass, B) brain mass and C) EQ



* estimates without fossils used a constant variance model, estimates including fossil used a directional model where there was support for a significant directional trend. In both cases EQ was estimated from ancestral state reconstructions of brain and body mass.

4. Figure S2: Posterior distribution of ancestral state reconstructions for node 1, the last common ancestor of Cetacea and Hippopotamidae



5. Effects of excluding selected taxa

Analyses using only extant species do not include any temporal variance in phenotypic diversity, however, the fossil record is an incomplete catalogue of past diversity. This is particularly true when excellent preservation is required to extract the trait of interest. All comparative analyses are dependent on the trait data, a phylogenetic hypothesis, and a model of character evolution. Therefore it is possible that additional extinct species, if added to an analysis such as this, could lead to different results if the fossils extend the range of phenotypic variation or add new temporal information about the course of evolution on particular branches. Our dataset lacks, for example, extinct mysticetes which vary greatly in size (Pyenson & Sponberg, 2011). To gain some insights into the sensitivity of our results, we repeated several analyses after removing selected key taxa. We tested for the presence of directional trends across all cetaceans following sequential removal of:

- Hippopotamidae, the outgroup, which may affect results if the mode of trait evolution in this clade differs greatly from that in Cetacea.
- *Dalanistes* and *Rodhocetus* the two most basal cetaceans in our dataset. It is possible these species could have a dominant effect on estimating the strength of any directional trend in a trait's evolution.
- *Basilosaurus*, a genus of large bodied stem cetaceans.
- All extant mysticetes. It is conceivable that these extremely large bodied species have a dominant effect on trait reconstructions at some nodes, particularly the last common ancestor of crown cetaceans, which would affect changes in trait values at adjacent nodes

In addition we repeated the test for directionality within odontocetes after excluding *Simocetus* and *Xenorophus*, the two most basal extinct odontocetes.

After removing selected taxa and re-estimating the best fitting model of evolution we subsequently estimated ancestral state reconstructions for five key nodes; the last common ancestor of Cetacea and Hippopotamidae (Figure 1, node 1), the last common ancestor of crown cetaceans (node 9), the last common ancestor of crown mysticetes (node 10), the last common ancestor of crown odontocetes (node 22) and the node preceding the stem lineage of extant

Delphinoidea (node 38). In two cases the exclusion of taxa removed one or more ancestral nodes from the phylogeny. Exclusion of hippos eliminated node 1, exclusion of mysticetes removed nodes 9 and 10. In the latter case we were interested in whether the mysticetes have a strong effect on nodes surrounding the origin of odontocetes as it is possible the reduction in body size observed at the origin of odontocetes is due to the effects of extant mysticetes leading to over-estimates of trait values at node 9. We therefore estimated the ancestral state at nodes 8 and 15, the two nodes surrounding the missing node 9.

Tests of directionality

In all cases the results presented in the main text are supported following the removal of selected key taxa (see supplementary spreadsheet, Table S5); both brain and body mass show a directional trend to increase through time (log-Bayes Factor > 2), but EQ does not. Unsurprisingly given their large size, removing all extant mysticetes has the strongest effect on the strength of evidence for a directional trend. This is because both extant and extinct taxa contribute to the estimation of the directional parameter, removing the largest species therefore affects the estimated relationship between size and root-to-tip branch length.

Ancestral state reconstructions

For the most part the removal of selected taxa has limited effects on the ancestral state reconstructions of brain and body mass (see supplementary spreadsheet, Table S6). For log(body mass) the average percentage difference from the estimate obtained with the full dataset was -0.322%, and the average absolute difference 1.196%. For log(brain mass) these percentage differences were 0.070% and 1.018% respectively. The largest percentage differences were observed for node 1, but across all treatments the range of estimates (12kg to 43kg) does not lead to conflicting conclusions. Similarly the removal of selected taxa generally affected ancestral state reconstructions at different nodes in the same direction, meaning estimated changes between nodes are relatively stable.

One notable, and perhaps surprising, aspect of our results was that on the stem lineage of Odontoceti body mass underwent a reduction in size, whilst brain mass did not. It is conceivable that this effect could be produced by extant mysticetes inflating the estimated body mass of the last common ancestor of crown cetaceans. However, when all extant mysticetes are excluded from the dataset the ancestral state reconstructions vary by less than 1% on average for both brain and body mass. Estimated changes in $\log(\text{body mass})$ between nodes 8 and 22 are -0.620 including all data and -0.563 excluding mysticetes. For $\log(\text{brain mass})$ these values are +0.089 and 0.+096 respectively, and for $\log(\text{EQ})$ they are +0.504 and +0.473. Hence the inferred allometric decoupling of brain and body mass evolution and associated increase in EQ at the origin of odontocetes is robust even to the exclusion of all extant mysticetes.

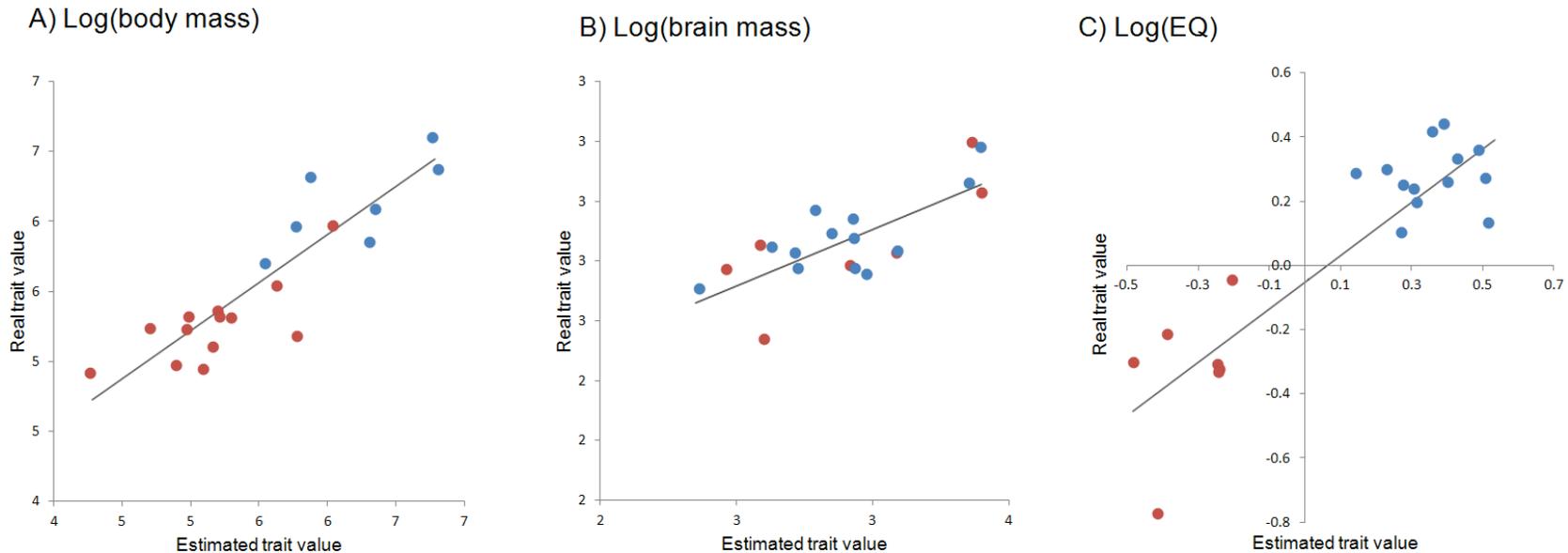
Excluding key fossil taxa may influence the ancestral state estimates at the nodes that these fossils are immediately connected to, but provided there is sufficient information to estimate the directional parameter of the trait evolution model, the effects of removing particular fossils appears to be minimal. Indeed, in a recent study Slater et al. (2012) combined simulations and empirical data to provide strong evidence that the integration of any fossil taxa, even a single species, is better than none at all because including information on the temporal distribution of traits helps uncover the best supported model of evolution.

Estimates for ancestral EQ values are more variable than brain and body masses. This is unsurprising as EQ is a composite measure derived from two reconstructed traits which may be affected in different directions by the removal of a particular species. On average the absolute percentage differences between the results obtained using the full dataset and those obtained excluding one or selected taxa vary by 17%. This is partially inflated by some instances where the the difference in results is particularly large. The median percentage difference is 8%. Removal of *Dalanistes* and *Rodhocetus* had the largest average affect on EQ estimates, but this is predominantly due to the estimate at node 1 which is caused by a lower estimated body mass and a slightly higher estimated brain mass. Despite these differences we again find that the major patterns and processes reported in the main text are stable to the exclusion of various fossil taxa.

6. Estimating extinct data to gauge model accuracy

Organ *et al.* (2007) suggested a simple method for checking the internal consistency of evolutionary models derived using Bayes Traits. Remove each species in turn, re-build the best supported model of evolution, and estimate the tip value of the missing species. We carried out this process for all extinct species in our dataset (see supplementary spreadsheet, Table S7). Across all taxa, estimated brain and body mass values were strongly correlated with the empirical data (Figure S3; body mass: $t_{18} = 8.157$, $p < 0.001$, $R^2 = 0.887$; brain mass: $t_{18} = 5.943$, $p < 0.001$, $R^2 = 0.755$; EQ: $t_{18} = 9.560$, $p < 0.001$, $R^2 = 0.887$). For log(body mass) the average absolute percentage error was 4.8% and for brain mass it was 6.4%. The range of percentage errors are reasonably evenly distributed around zero with mean errors of 1.1 and -0.48% for body and brain mass respectively. As observed above, estimates of EQ are more sensitive as they are derived from the independently estimated values for brain and body mass and therefore compound error.

Figure S3. Correlations between empirical and estimated data. Blue dots are extinct odontocetes, red dots are other extinct species.



7. Comparison between ancestral state estimates at key nodes the fossil record

Our taxonomic sample included all extant and extinct cetaceans for which estimates of body mass, brain mass, and phylogenetic position (as determined by matrix-based phylogenetic analyses) are available. Given the difficulty in obtaining brain size estimates in extinct taxa, we are only able to include a small fraction of the fossil record. For this reason, some of the trends we detected in cetacean evolution could be contradicted by the body masses of the taxa excluded (but see sections 5 and 6 of this supplement that suggest our results are reasonably robust to taxon sampling). In this section we discuss published body size estimates for some fossil cetaceans as well as present some new estimates, and then compare those to body size estimates we obtained for three key nodes. We hope that this section will spur additional efforts to describe fossil specimens, publish additional measurements, and collect CT data for estimating brain size. One caveat to this section is that such comparisons are not a substitute for actual analyses; the effect on ancestral node estimates of including additional taxa to our phylogenetic hypothesis by branches of varying lengths cannot be easily predicted.

a) The earliest cetaceans

In terms of statistical confidence, the most challenging node to estimate is at the base of the tree, the common ancestor of Hippopotamidae + Cetacea (Figure 1: node 1), because this node is not informed by data from more basal branches. This particular problem could be alleviated by adding additional semi-aquatic and terrestrial artiodactyls to our dataset, but this would only shift the problem to a more basal node. Our goal is to test evolutionary trends in brain and body size in Cetacea, not Artiodactyla as a whole.

We estimate that the common ancestor of Hippopotamidae + Cetacea (node 1) had a body mass of 43.4 kg, which is smaller than the most basal cetacean we sampled (*Dalanistes ahmedi*: 750 kg) and the two hippopotamids included (*Hippopotamus amphibius*: 2000 kg, *Choeropsis liberiensis*: 275 kg). Although our model was probably correct in estimating a much smaller body mass for this node as compared to adjacent terminal taxa, fossils not included in our study (because they lack brain size estimates) suggest the actual size of this ancestor is much smaller. Recent phylogenetic analyses agree that the most basal cetacean family is Pakicetidae and that

the immediate sister-group to Cetacea is the Raoellidae (Geisler & Uhen 2005; Thewissen et al. 2007; Geisler & Theodor 2009; Gatesy et al. 2013). We applied regression equations to skeletal measurements of pakicetids and raoellids to estimate their body masses. The first regression is based on extant cetaceans and predicts body mass based on the width across the occipital condyles (Marino et al. 2004). Specifically:

$$\log(\text{body mass [kg]}) = 3.814 * \log(\text{width across condyles [mm]}) - 5.345$$

The second and third equations are based on a sample of extant artiodactyls and utilize different measurements of the postcranial skeleton (Scott, 1990).

$$\log(\text{body mass [kg]}) = 2.6454 * \log(\text{distal transverse width of humerus [cm]}) + 0.2538$$

$$\log(\text{body mass [kg]}) = 2.4768 * \log(\text{distal articular width of radius [cm]}) + 0.4677$$

We consider estimations of body sizes in pakicetids and raoellids to be quite uncertain due to the lack of close extant relatives that have similar body forms and habits. Parsimony optimizations of habitat (Gatesy et al. 2013) and pachyosteosclerosis of limb bones (Thewissen et al. 2007) suggest that raoellids and pakicetids spent considerable time in freshwater environments. Based on these inferences, the regression based on a sample of extant cetaceans (i.e. Marino et al. 2004) might be most appropriate. However, both raoellids and pakicetids had large hind limbs similar in proportion to those of extant artiodactyls, suggesting that regressions based on postcranial measurements of terrestrial artiodactyls would be more reliable. We have used both sets of equations to bracket the possible body sizes of the earliest cetaceans and their close relatives.

Widths across the occipital condyles yield body mass estimates for pakicetids that vary from approximately 8 to 18 kg (Table S8). One such taxon, *Nalacetus ratimitus*, is estimated to have a body mass of 10.9 kg, whereas the radius of a separate individual referred to that taxon yields a similar estimate of 7.24 kg (distal end of radius is 14.4 mm, Madar, 2007). These estimates are considerably smaller than a body mass of 43.4 kg that we estimate for the common ancestor of Hippopotamidae + Cetacea, and raoellids are even smaller than pakicetids. One specimen of the raoellid *Indohyus* (RR 149, Cooper et al., 2012) has distal humeral width of 16.3 mm, which using the regression equation listed above from Scott (1990), yields a body mass estimate of just 6.5 kg. Thewissen et al (2007) give the ln of the width across the occipital condyles from another

specimen of *Indohyus* (2.83, RR 208), which when converted to its original measurement of 16.94 mm and then used with the regression equation of Marino et al. (2004), yields a diminutive body mass 0.2 kg. That measurement was made in error, and a new estimate derived by doubling the length of the right side gives a width across the condyles of 31.8 mm (Thewissen, pers. comm.). The body mass implied by this measure is still small, only 2.4 kg, but 10 times the initial estimate. Another specimen of *Indohyus* (RR 207) has an estimated width across the condyles of 25 mm (Thewissen, pers. comm.) and an estimated body mass of 1.1 kg. Such small body mass estimates may indicate that a regression based on extant cetaceans, all of which are much larger than *Indohyus*, is inaccurate when applied to a much smaller and differently proportioned mammal.

The above body size estimates of pakicetids and raoellids indicate that our analysis may overestimate the body mass of the common ancestor of Hippopotamidae + Cetacea. However, given that this is the most basal node in our tree, a smaller body size would only reinforce the trend towards increasing body size we have detected across the entire cetacean clade. Furthermore, including body size estimates from these fossils in actual analyses is problematic for several reasons. First, as described above, pakicetids and raoellids straddle the transition from terrestrial habitats to aquatic habitats, thus it is unclear what extant taxa and what skeletal measurements should be used to develop regression equations to estimate their body sizes. Second, published skeletons of pakicetids and raoellids are composites of multiple individuals and taxonomic identities were inferred based on size, morphology, isotopic composition, and geologic co-occurrence (Thewissen et al 2001, 2007; Madar 2007; Cooper et al. 2012). Thus in some cases postcranial elements have only been identified to genus, and in others it is possible that identifications will change if associated or articulated fossil material is found.

b) The origin of crown cetaceans and stem odontocetes

Although we detected overall trends across Cetacea of increasing body and brain size, some of the largest increases in EQ occurred in the portion of the tree that includes the common ancestor of crown cetaceans and the common ancestor of all odontocetes (Figure 1: nodes 9 and 15). Here, increases in EQ were predominantly caused by exceptions to the overall trends we

detected; body size decreased and brain size either stayed the same or slightly increased. Given the importance of this part of the tree for understanding how the high EQ of most extant odontocetes originated, we briefly discuss the extinct taxa not included in our study for the reasons described above and compare body size estimates of these taxa to the estimates we derived for two nodes: 1) the most recent common ancestor of crown cetaceans (node 9) and 2) the most recent common ancestor of *Xenorophus* and all extant odontocete (node 15).

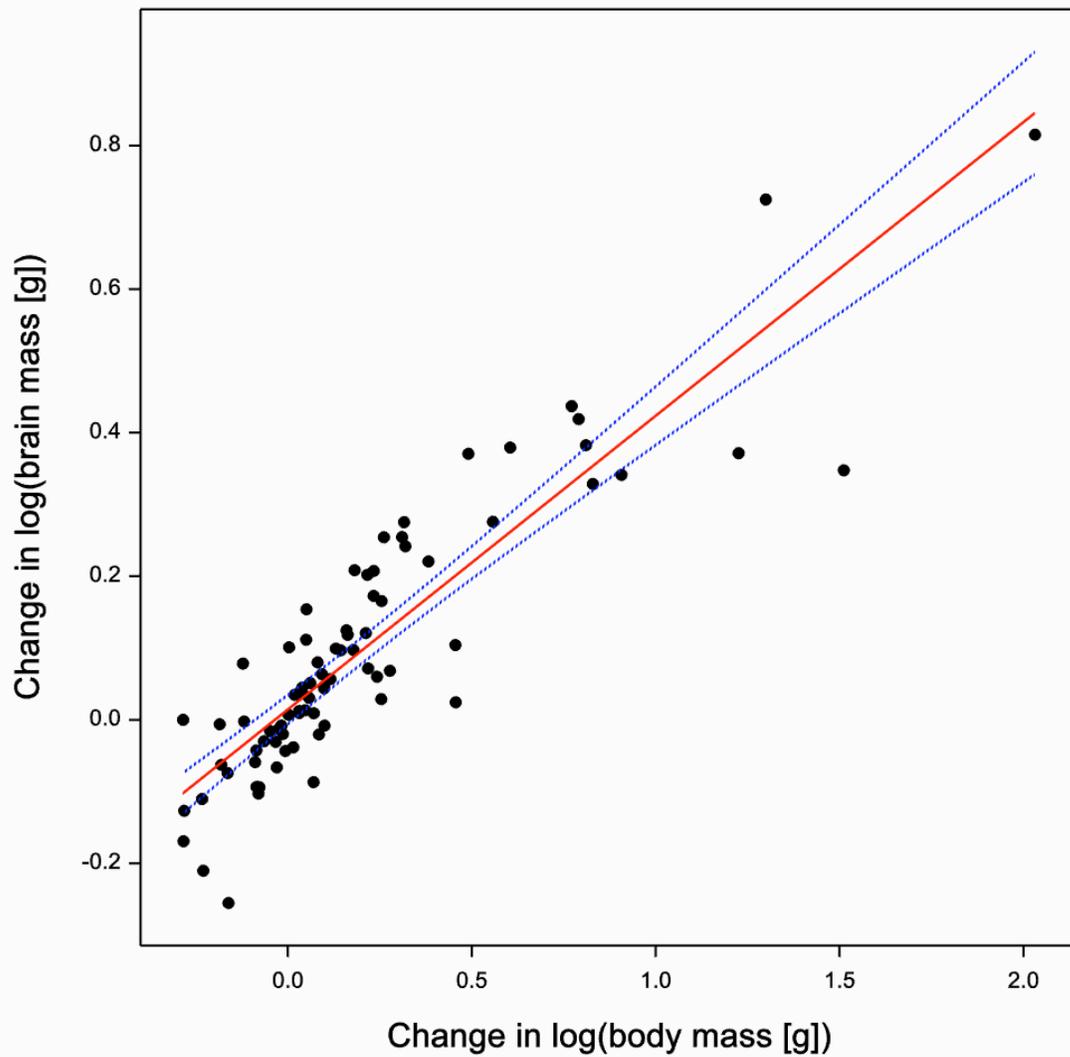
In general, body mass estimates for basal stem mysticetes appear to be consistent with our estimate for the body mass of the ancestral neocete (i.e. 166.6 kg). For example, using the equation of Marino et al. (2003, 2004), the body mass of the basal mysticete *Mammalodon colliveri* is approximately 190 kg and other toothed mysticetes that are somewhat more removed from the base of Neoceti (Deméré & Berta 2008; Deméré et al. 2008; Fitzgerald 2009) have body sizes that range from 117 to 184 kg (Table S8). The occipital condyles of another mammalodontid, *Janjucetus hunderi*, are crushed and only partially preserved, thus we are not able to estimate its body mass. However, other measurements suggest it had a similar body mass as *Mammalodon colliveri* (Fitzgerald 2009). One important exception is *Llanocetus denticrenatus*, from the late Eocene of Seymour Island, Antarctica (Mitchell 1989). The complex history of this taxon is discussed elsewhere (i.e. Fitzgerald 2009), and will not be repeated here, but the important point is that the skull of the holotype is mostly undescribed, cranial measurements have not been published, and thus we are not able to independently estimate its body mass. However, in a meeting abstract, Fordyce (2003) estimated *Llanocetus* to have had a body length exceeding 9 meters. Uhen (2004) developed an equation that relates body mass to skeletal length in extant cetaceans, and when applied to *Llanocetus* indicates that it had a body mass greater than 14,000 kg. Incorporation of *Llanocetus* into future analyses that model the evolution of body size will have to wait until more data are published on the skull, but this should be an imperative given its large body size, early geologic age (it is the oldest described crown cetacean and the only one known from the Eocene), and the fact that the holotype includes a well preserved endocast (Mitchell 1989). Whether its incorporation would increase the estimate for the body size of the ancestral crown cetacean is difficult to say given that its closest relatives, other mammalodontids such as *Mammalodon colliveri* (see above), are much smaller (Fitzgerald 2009).

Basal odontocetes that we did not include are generally much smaller than basal mysticetes; we estimate that *Archaeodelphis patrius* had a body mass of 19.6 kg, *Albertocetus meffordorum* was 50.8 kg, *Patriocetus kazakhstanicus* was 103.2 kg (Table S8), and two undescribed taxa, USNM 335502 and 256604, were estimated by Marino et al. (2004) to be 54.8 and 78.1 kg respectively. Our ancestral state estimates already show a decrease in body size from 166 to 130 kg between the common ancestor of crown cetaceans and the first node within Odontoceti, and it is possible that if the above odontocetes were included, the decrease in body size would be even more abrupt. However, the phylogenetic positions of many of these taxa are unclear, and their influence on estimates for the base of Neoceti and for the most recent common ancestor of *Xenorophus* and all extant odontocetes (i.e. node 15) would presumably diminish if they have a more apical position along the odontocete stem.

TS8: Measurements and body size estimates for select fossil cetaceans and raoellids. OCW = width across occipital condyles. BM = body mass. * = estimated measurement. RR = Ranga Rao collection.

Taxon	OCW (mm)	Body mass (g)	Log₁₀ (BM)	Reference for measurement
<i>Indohyus sp.</i> (RR207)	25.6*	1061.8	3.03	Thewissen pers. comm.
<i>Indohyus sp.</i> (RR208)	31.8	2428.0	3.39	Thewissen pers. comm.
<i>Pakicetus attocki</i>	52.47	16396.1	4.21	Numella et al (2007)
<i>Pakicetus inachus</i>	53.7	17911.1	4.25	Numella et al (2007)
<i>Ichthyolestes pinfoldi</i>	43.6	8091.0	3.91	Numella et al (2007)
<i>Nalacetus ratimitus</i>	47.1	10861.8	4.04	Numella et al (2007)
<i>Mammalodon</i>	100	191866.9	5.28	Fitzgerald (2009)
<i>Chonocetus goedertorum</i>	78	74378.7	4.87	Barnes et al (1994)
<i>Ashorocetus eguchii</i>	88	117830.3	5.07	Barnes et al (1994)
<i>Morawanocetus yabukii</i>	89	123019.4	5.09	Barnes et al (1994)
<i>Aetiocetus tomitai</i>	93	145476.9	5.16	Barnes et al (1994)
<i>Aetiocetus cotylalveus</i>	91	133901.0	5.13	Barnes et al (1994)
<i>Aetiocetus weltoni</i>	99	184651.4	5.27	Barnes et al (1994)
<i>Aetiocetus polydentatus</i>	91	133901.0	5.13	Barnes et al (1994)
<i>Archaeodelphis patrius</i>	55	19622.0	4.29	Uhen (2008)
<i>Albertocetus meffordorum</i>	70.6	50855.9	4.71	Uhen (2008)
<i>Patriocetus kazakhstanicus</i>	85	103229.5	5.01	Dubrovo & Sanders (2000)

8. Figure S3: Regression between changes in cetacean brain and body mass



Red line indicates the regression line ($t_{81} = 14.670$, $p < 0.001$, slope = 0.427), the dotted blue lines indicate the 95% confidence intervals.

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