



# Macroevolution of Toothed Whales Exceptional Relative Brain Size

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## Abstract

Toothed whales (Odontoceti, Cetacea) are well-known for their ability to produce complex vocalizations, to use tools, to possess self-recognition, and for their extreme behavioural plasticity. The toothed whale intelligence is said to compete with that of primates, so does their extremely large brain to body size ratio. Common explanations for the acquisition of such large brains over the evolutionary time (encephalization) in toothed whales range from their demanding, complex social lives, to their feeding habits, to echolocation. Yet, several studies found no macroevolutionary trend in Odontoceti encephalization, which casts doubts on its selective advantage. We applied a recently developed phylogenetic comparative method to study macroevolutionary trends in relative brain size (RBS) and brain size evolutionary rates in cetaceans, comparing toothed whales to the other cetaceans and contrasting groups of species as ascribed to different feeding categories. We found that cetaceans as a whole followed a trend for increased encephalization over time, starting from small-brained archaeocete ancestors. Toothed whales do not show this same trend in RBS but have possessed larger RBS than any other cetacean ever since the beginning of their existence. The rate of RBS evolution in Odontoceti is significantly slower than in other Cetacea and slower than the rate of Odontoceti body size evolution. These results suggest that toothed whales' history is characterized by high and conservative relative encephalization. Feeding lifestyle does not explain these patterns, while the appearance of echolocation within stem group Odontoceti remains a viable candidate for them.

**Keywords** Encephalization · Relative brain size · Cetaceans · Odontoceti · Mysticeti

## Introduction

Cetaceans originated some 53 million years ago (Mya) from land mammal ancestors to be probably found among raoellids (Thewissen et al. 2007; McGowen et al. 2014). The morphological transition from the terrestrial to the aquatic

lifestyle is attested by numerous Eocene species, commonly grouped under the heading “archaeoceti”. This wastebasket taxon (Milinkovitch 1995) includes a variety of ecologically dissimilar forms, from terrestrial species wading shallow waters in search of food (*Pakicetus*, *Ambulocetus*, *Remingtonocetus*), to fully aquatic taxa (*Basilosaurus*, *Dorudon*). Modern cetaceans (*Neoceti*) originated from an archaeocete ancestor close to *Dorudon* in the late Eocene (Slater et al. 2010; Marx and Fordyce 2015). The diversification of Neoceti has long been described as a case of adaptive-radiation, spurred by both ecological opportunities (Clementz et al. 2006) and key innovations (i.e. baleens in mysticetes, echolocation in odontocetes, Fordyce 1992). However, such an ‘explosive’ phase is more consistent with early morphological differentiation prompted by dietary adaptations (Slater et al. 2010), following major changes in paleoceanographic currents (Steeman et al. 2009).

Neoceti include two monophyletic sister taxa. Mysticetes (baleen whales) are among the largest species ever lived on Earth, with body size spanning from 3 (pigmy right whale,

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*Caperea marginata*) to 170 (the blue whale, *Balaenoptera musculus*) tons. All extant Mysticeti are adapted to bulk filter feeding (Berta et al. 2016). The early radiation of mysticetes (ca 39 to 28 Mya) coincides with the onset of the Antarctic Circumpolar Current (Steeman et al. 2009; Marx and Fordyce 2015), which possibly favoured the diversification of toothed forms (i.e. *Mammalodontidae* and *Aetiocetidae*) and the emergence of Chaemysticeti (toothless whales). Around the Oligocene/Miocene boundary (23 Mya), toothed Mysticeti begun to vanish, to be replaced by filter feeders (FFs) provided with baleen.

The evolutionary history of Odontoceti (toothed whales) is characterized by the radiation of Delphinida around 11 Mya (Steeman et al. 2009). This latter clade is the most speciose among cetaceans and includes species living in the sea and freshwater taxa, ranging from the poles to the tropics. Living odontocetes span in body size from 54 kg (the vaquita, *Phocena sinus*) to 57 tons (sperm whale, *Physeter macrocephalus*). They are active predators, feeding near shore (like dolphins and killer whales occasionally do) up to 2 km in depth (like sperm whales). Their diet is mainly composed by fish and cephalopods, but they also feed on crustacean, bivalves, and other mammals (Berta et al. 2007). Odontocetes also differentiate among each other in the way they catch their prey. Suction feeders (SFs) have a rounded snout and reduced dentition as they suck down the prey (usually cephalopods) into the mouth. Raptorial feeders hold their prey by strong and rapid closure of the jaws. Among raptors, the minimum prey size strongly relates to the skull length, as longirostrine (i.e. long-snouted) species can use their snout to catch small and agile prey (McCurry et al. 2017).

Toothed whales are commonly noted for their extraordinary behavioural plasticity. A number of studies indicate several species evolved learning, communication, social and cultural skills which have no parallel in non-human species (Rendell and Whitehead 2001), to the extent that they have been described as the ‘apes better than apes’ (Whiten 2001). Most odontocete cetaceans live in complex groups with extremely differentiated relationships, long-term bonds, cooperative networks and alliances (Connor 2007), which strongly depend on their ability to recognize and understand others, and to communicate with a wide range of vocal, visual, and behavioural signals (Reidenberg and Laitman 2004; Cancho and Lusseau 2006; Lusseau 2006). They are also able to recognize themselves and their own body parts (Herman et al. 2001; Reiss and Marino 2001), use tools (Krützen et al. 2005), and transmit learned behaviours (Rendell and Whitehead 2001).

The explanation for such astonishing abilities likely lies in cetacean brain size and complexity (Marino et al. 2007). Cetacean brains are the largest among vertebrates and are extremely gyrified as compared to the brain of terrestrial

mammals (Marino 2004). Anatomical investigations of Odontocete brains revealed their high encephalization quotient (EQ, the brain to body relative size, Jerison 1985; Gingerich 2015), and that the number of neocortical neurons and glial cells is consistently larger than in any other large-brained mammal species, including humans (Mortensen et al. 2014). It is commonly assumed that the large brain in odontocetes evolved in response to the cognitive demands associated to their social and behavioural complexity (Rendell and Whitehead 2001; Connor 2007; Wright et al. 2017). It was possibly accompanied by the acquisition of echolocation (Marino et al. 2004, 2007; Mortensen et al. 2014; Churchill et al. 2018) from the inception of the clade (Park et al. 2016) and persisted through the initial body size reduction the group underwent (Marino et al. 2004; Montgomery et al. 2016). However, some studies suggested the cetacean brain size is unrelated to cognitive abilities (Manger 2006, 2013). Others have shown that the higher relative brain mass in Odontoceti as compared to Mysticeti is just the result of higher rate of body size evolution in the latter, and that toothed whales relative brain mass did not increase through time. These results might imply the high EQ in odontocetes could be neutral with respect to cognitive abilities (Montgomery et al. 2013).

Herein, we investigate upon the timing, rate and trend of encephalization in odontocetes and cetaceans as a whole. We used a 89 species wide phylogeny inclusive of 33 fossil species, and their estimated brain volumes, and applied a recently developed phylogenetic comparative method (PCM, *RRphylo*, Castiglione et al. 2018) explicitly sought to compute evolutionary rates and phenotypes on the tree, and to locate shifts in the evolutionary rate by using paleontological phylogenies.

Traditional approaches to study the evolution of encephalization involve the calculation of the typical brain–body allometry (i.e. the EQ; or the residuals of brain to body mass regression, RBS), which can be problematic as they do not represent a true evolutionary approach (Harvey and Pagel 1988; Shultz and Dunbar 2010), and because residuals have undesirable statistical properties under both non-phylogenetic and phylogenetic contexts (Freckleton 2002, 2009). Therefore, we developed a new version of *RRphylo* to perform phylogenetic multiple regression between brain size and body size.

## Materials and Methods

The cetacean phylogenetic tree is an informal supertree (Online Resource 5) assembled from the backbone phylogenies in Montgomery et al. (2013) and Marx and Fordyce (2015). The phylogenetic position of individual species, along with the scientific references for such data are

available as Online Resource 2. The tree includes 89 species, 33 of them are extinct (9 archaeocetes, 23 odontocetes, 1 mysticete).

Body size estimates were taken from the literature as specified in Online Resource 3. Data were in grams and converted in ln grams before the analyses.

Brain volumes (cm<sup>3</sup>) and size (g) were taken from several sources as indicated in Online Resource 3. Brain volumes were converted in brain sizes using the mean density of brain tissue = 1.036 g/cm<sup>3</sup> (Navarrete et al. 2018).

Estimates of brain volume obtained via allometric equations were excluded. We did not estimate brain mass from endocranial volume since part of the skull cavities is occupied by tissues other than brain in Cetacea (Ridgway et al. 2016).

We tested for the potential influence of diet on encephalization which has been reported for a number of animal groups, including humans (Aiello and Wells 2002), primates (DeCasien et al. 2017), carnivores (Gittleman 1986), bats (Eisenberg and Wilson 1978), small mammals (Mace et al. 2009), and possibly marsupials (Weisbecker et al. 2015).

We ascribed each species to a feeding category. We used two alternative categorization schemes. First, we partitioned species into FFs, SFs, and raptorial feeders (R), according to the feeding category scheme adopted in Uhen (2004), Johnston and Berta (2010) and Berta et al. (2016). In the second feeding category scheme, we further divided raptorial species into brevirostrine (BR), and longirostrine (LR), forms, which are known to differ from each other in terms of prey selection (McCurry et al. 2017). To assign a species to either BR or LR we used the relative length of the rostrum (from the tip of the muzzle to the rearmost point of the fronto-nasal suture (Bianucci and Landini 2002, see Online Resource 3).

To compare brain size among different feeding categories while keeping into account the allometric effects and group size, we computed the estimated marginal means (the equally weighted group mean predictions) of brain versus body size regression per group and performed pairwise comparisons between feeding category groups by using the package emmeans (Lenth 2018).

### Phylogenetic Multiple Regression with *RRphylo*

We applied a PCM available in the R package *RRphylo* (Raia et al. 2019). This method, *RRphylo* (Castiglione et al. 2018), performs phylogenetic ridge regression (Kratsch and McHardy 2014) by using a phylogenetic tree and data. This PCM returns a vector of phenotypic evolutionary rates for each branch of the tree and a vector of ancestral state estimates for each node. *RRphylo* works by penalizing the evolutionary rate coefficients as to minimize the rate variation within clades (Castiglione et al. 2018).

Since rates are in fact phylogenetic ridge regression coefficients, their magnitude depends on the absolute values of the phenotypes being regressed (Castiglione et al. 2018), that means large phenotypic values will originate large rates even with small phenotypic change. To standardize the rates, under *RRphylo* it is advisable to use the phenotype itself as a covariate. For instance, it is possible to use body size as a covariate to calculate the mass-specific rate of body size evolution for each branch in the tree. In the case of brain size, the evolutionary rates would strongly depend on allometric effects (so that large-brained species would show large evolutionary rate values, whether or not their brains are larger than expected by their body size). Using EQs or the residuals of the brain to body size regression (RBS) helps fixing the problem, but ratios and residuals have undesirable statistical properties that make their use questionable (Freckleton 2002, 2009). Because of this, we developed a new version of *RRphylo*, that allows calculating the evolutionary rates from a multiple regression (in the present context, brain size is the response variable, the phylogeny and body size are the predictors).

In the multiple regression *RRphylo* version rates are calculated as:

$$\hat{\beta} = (L^T L' + \lambda I)^{-1} L^T y$$

where  $L'$  is the tip to node path of branch lengths matrix (which represents phylogeny as a predictor) supplemented with body size as its last column (representing body size as the additional predictor) and  $y$  is the vector of species brain sizes. Lambda ( $\lambda$ ) is the normalization factor optimized to avoid abnormal rate values within clades and  $I$  is the identity matrix. This way, the vector of rates  $\hat{\beta}$  is calculated for all the branches in the tree and the last element of  $\hat{\beta}$  represents the partial phylogenetic ridge regression coefficient of the additional predictor (here body size). We tested the appropriateness of this procedure by virtue of simulation experiments and demonstrated multiple *RRphylo* correctly represents an unbiased estimator of ancestral states and rates just as *RRphylo* (Online Resource 6).

We applied *RRphylo* on body size and the multiple *RRphylo* version on brain size in cetaceans. Absolute rate values computed under *RRphylo* were used to search for evolutionary rate shifts by using the function *search.shift* in the *RRphylo* package. This function scans the distribution of rate values on the tree branches for possible changes in mean rates under two different conditions: “clade” and “sparse”. Under the former, the average absolute evolutionary rate of specific clades is compared to the rest of the tree by means of randomization. Under the “sparse” condition, *search.shift* tests whether tips under a given state evolve at a different average rate as compared to the rest of the tips. Significance is assessed

via randomization (of rate values among states or clades either, Castiglione et al. 2018). The function may search for rate shifts in magnitude at specified nodes (or states) in the tree, or run into the ‘auto.recognize’ mode. Under the latter (which we applied here) instances of significant rate shifts are located on the tree. We applied *search.shift* on body and brain size evolutionary rates. We also used *search.shift* under the “sparse” setting to test for difference in brain size evolutionary rates among dietary categories.

After testing for the existence of rate shifts, we applied a newly developed RRphylo function, *search.trend* (Castiglione et al. 2019), which regresses phenotypes and (absolute) evolutionary rate values against their distance from the tree root, assessing the existence of macroevolutionary trends in rates of phenotypic evolution and phenotypic values. The significance of both regression slopes is tested by comparing them to a family of 100 regression slopes generated according to the Brownian motion model of evolution. As with *RRphylo*, we modified *search.trend* to deal with evolutionary rates derived from a multiple phylogenetic ridge regression. In particular, for each simulated Brownian motion phenotype the new *search.trend* adds a predictor as with the original multiple regression *RRphylo* design. The predictor is simulated and then modified to have the same correlation structure with the response variable as the original data. This is achieved by transforming both the simulated response and the simulated predictor by using Cholesky decomposition.

The *search.trend* function is able to identify ‘trends’ in rates and phenotypic ‘drift’ in specific clades, and to compare them to the rest of the tree. For each regression, estimated marginal means are contrasted between the focal clade and the rest of the tree by using the functions embedded in the package *emmeans* (Lenth 2018).

We applied *search.trend* on body and brain size data and rates, and contrasted both the crown group and the stem group (extinct representatives of the clade with no living descendants) to the rest of the cetacean tree. The functions are available on CRAN (<https://cran.r-project.org/web/packages/RRphylo/index.html>). The multiple regression versions of *RRphylo* and *search.trend* is available at <https://github.com/pasraia/RRphylo>.

Because of the long-standing tradition of using EQ and relative brain size (RBS, the residuals of brain to body size allometric equation) in studying the evolution of encephalization in mammals, we used the equations in Montgomery et al. (2013) to compute EQ, and calculated RBS as the residuals of the linear regression between (ln) brain volume and (ln) body size. Macroevolutionary trends in evolutionary rates and phenotypes for EQ and RBS were calculated and presented as Electronic Supplementary Information (Online Resource 1).

## Accounting for Phylogenetic Uncertainty

To assess for potential biases as introduced by phylogenetic uncertainty, we developed and applied the newly implemented RRphylo function *overfitRR* to test the effect of sampling, tree topology and branch lengths on results produced by *search.trend* and *search.shift*. This function randomly removes a number of tips corresponding to 25% of the tree size and swaps species phylogenetic position (thereby accounting for sampling effects) by using the RRphylo function *swapONE*. Under *swapONE*, each tip might change its position on the tree by up to two nodes. For instance, a topology of the kind ((A, B), C) might change to ((C, B), A) or ((A, C), B). In addition, each node might change in age in between the age of its ancestor and the age of its daughter node. We set one tenth of the tips to be swapped across nodes and one tenth of the nodes to be changed in age at each iteration. Then, it performs *search.trend* and *search.shift* on pruned tree and data. The procedure is repeated 100 times and the percentage of significant results returned. In this case, we specified the Odontoceti clade to be tested for temporal trends in phenotypic (body and brain size) mean and rates. It must be noted that with such modest level of swapping the original topology (which is used as the reference for producing the random trees) is not altered significantly. This helps avoiding testing unreliable topological arrangements which could be unrealistic or otherwise unsupported in the scientific literature.

## Results

### Body Size

By applying *search.shift* under the ‘auto.recognize’ mode we found a positive and significant shift in body size evolutionary rates pertaining to the clade including Platanistidae and Eurhinodelphinidae (average rate difference = 0.941,  $p = 0.005$ ).

We did not find evidence for increase in body size (Cope’s rule) in Cetaceans as a whole ( $p = 0.470$ , Table 1A). The same is true of toothed whales ( $p_{\text{crown}} = 0.370$ ,  $p_{\text{stem}} = 0.490$ , Table 1B). Unsurprisingly, estimated marginal means in body size in Odontoceti are significantly smaller than for the other species (Table 1B).

The temporal trend in absolute evolutionary rate was not significant for the entire clade ( $p = 0.370$ , Table 1A). A trend for increased rates is present in crown Odontoceti ( $p = 0.004$ ) but not for the stem group ( $p = 0.089$ , Table 1C). The estimated marginal means of the rates of body size evolution versus time regression in Odontoceti are no different from the rest of the cetacean tree ( $p_{\text{crown}} = 0.118$ ,  $p_{\text{stem}} = 0.378$ , Table 1C).

**Table 1** Evolutionary trends through time for body size, for Cetacea (A) and Odontoceti (B–C)

(A) Temporal trends in Cetacea body size				
	Slope		p-value	
Body size	0.001		0.470	
Body size evolutionary rates	0.035		0.370	
(B) Temporal trends in Odontoceti body size				
	Slope	p-value	Marginal means difference	p marginal means difference
Crown Odontoceti	0.005	0.370	−0.359	<0.001
Stem Odontoceti	0.001	0.490	−0.402	<0.001
(C) Temporal trends in Odontoceti body size evolutionary rates				
	Marginal means difference	p marginal means difference	Slope difference	p slope difference
Crown Odontoceti	−0.102	0.118	0.013	0.004
Stem Odontoceti	−0.058	0.378	0.006	0.089

*Slope regression slope*, *p-value* significance level assessed by contrasting the real slope to random slope values derived from Brownian motion simulations, *p marginal means difference* the statistical significance for the difference in marginal means between Odontoceti to all other Cetaceans, *slope difference* the difference between regression slopes for Odontoceti contrasted to the slope obtained for all other Cetaceans, *p slope difference* p-value for the difference in slopes between Odontoceti and the other Cetaceans

**Table 2** Clades showing significant shifts in brain size evolutionary rates

	Average rate difference	p-value
Platanistidae + Eurhinodelphinidae	−0.090	0.002
Physeteroidea	−0.089	0.009
Balaenopteridae	0.224	<0.001
Ziphiidae	0.117	0.014

*Average rate difference* difference in average rates between the focal clade and the rest of the tree, *p value* significance level for the difference as assessed by means of randomization

## Brain Size Evolution

By scanning the phylogeny for significant shifts in brain size evolutionary rates, we found significantly higher absolute rates for Balaenopteridae and Ziphiidae, and significantly smaller rates pertaining to the clades including Platanistidae and Eurhinodelphinidae, and Physeteroidea (sperm whales), respectively (Table 2).

Cetaceans as a whole showed nearly significant (at the nominal alpha level = 0.05) trend for increasing brain size over time ( $p = 0.060$ , Table 3A). The rate of brain size evolution significantly increased through time ( $p < 0.001$ , Table 3A).

There was no evidence for a macroevolutionary trend in brain size in stem Odontoceti ( $p = 0.260$ , Table 3B; Fig. 1) nor for crown Odontoceti ( $p = 0.230$ , Table 3B; Fig. 1). However, both stem and crown group Odontoceti

showed significantly higher estimated marginal means in the brain size versus time regression as compared to the other cetaceans (Table 3B; Fig. 2).

The rate of brain size evolution did not change over time in toothed whales, either considering the stem ( $p = 0.297$ ) or crown ( $p = 0.092$ ) Odontoceti (Figs. 1, 2; Table 3C). However, estimated marginal means in brain size evolutionary rates for both stem and crown Odontoceti are significantly lower than for other cetaceans (Table 3C; Fig. 2).

## Feeding Category

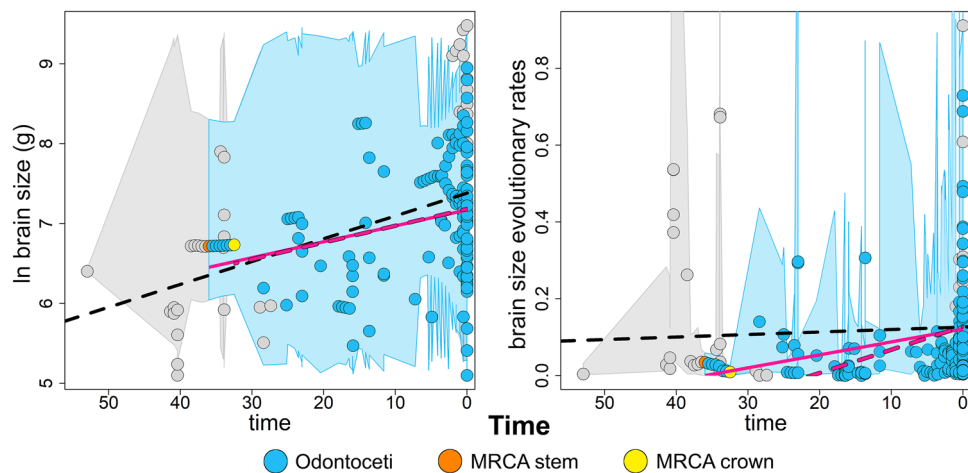
We computed differences between feeding categories in terms of brain size considering either 3 (Feed3) or 4 (Feed4) different categories (see Online Resources 1, 4 for results for RBS and EQ). The group comparison produced no significant differences between feeding categories (Table 4B–C).

Comparing rates by state by means of *search.shift* performed under “sparse” condition for the Feed3 category, we found negative and significant shifts in rates of brain size evolution in raptorial feeders compared to the rest of the tree and to filter feeders ( $p = 0.023$  and  $0.024$  respectively, Online Resource 4, Table S5). The same analysis performed on Feed4 indicates filter feeders show significant higher rates than longirostrine species ( $p = 0.022$ , Online Resource 4, Table S6).

**Table 3** Evolutionary trends through time for brain size, for Cetacea (A) and Odontoceti (B–C)

(A) Temporal trends in Cetacea brain size				
	Slope		p-value	
Brain size	0.007		0.060	
Brain size evolutionary rates	0.018		<0.001	
(B) Temporal trends in Odontoceti brain size				
	Slope	p-value	Marginal means difference	p marginal means difference
Crown Odontoceti	0.005	0.230	0.108	<0.001
Stem Odontoceti	0.005	0.260	0.156	<0.001
(C) Temporal trend in Odontoceti brain size evolutionary rates				
	Marginal means difference	p marginal means difference	Slope difference	p slope difference
Crown Odontoceti	−0.126	<0.001	0.004	0.092
Stem Odontoceti	−0.132	<0.001	0.002	0.297

*Slope* regression slope, *p-value* significance level assessed by contrasting the real slope to random slope values derived from Brownian motion simulations, *p marginal means difference* the statistical significance for the difference in marginal means between Odontoceti to all other Cetaceans, *slope difference* the difference between regression slopes for Odontoceti contrasted to the slope obtained for all other Cetaceans, *p slope difference* p-value for the difference in slopes between Odontoceti and the other Cetaceans



**Fig. 1** Plots of phenotypic values (left) and evolutionary rates (absolute values, right) versus time for brain size. Gray dots represent cetacean species not belonging to Odontoceti. Regressions for the whole tree are represented by the black dashed lines. Regressions for stem group Odontoceti are indicated by the pink solid lines. Regression for crown group Odontoceti are indicated by the pink dashed lines. The

shaded areas represent the 95% confidence intervals for rates or phenotypes at specific branches as generated under trendless data. *MRCA stem* most recent common ancestor of stem group Odontoceti, *MRCA crown* most recent common ancestor of crown group Odontoceti (Color figure online)

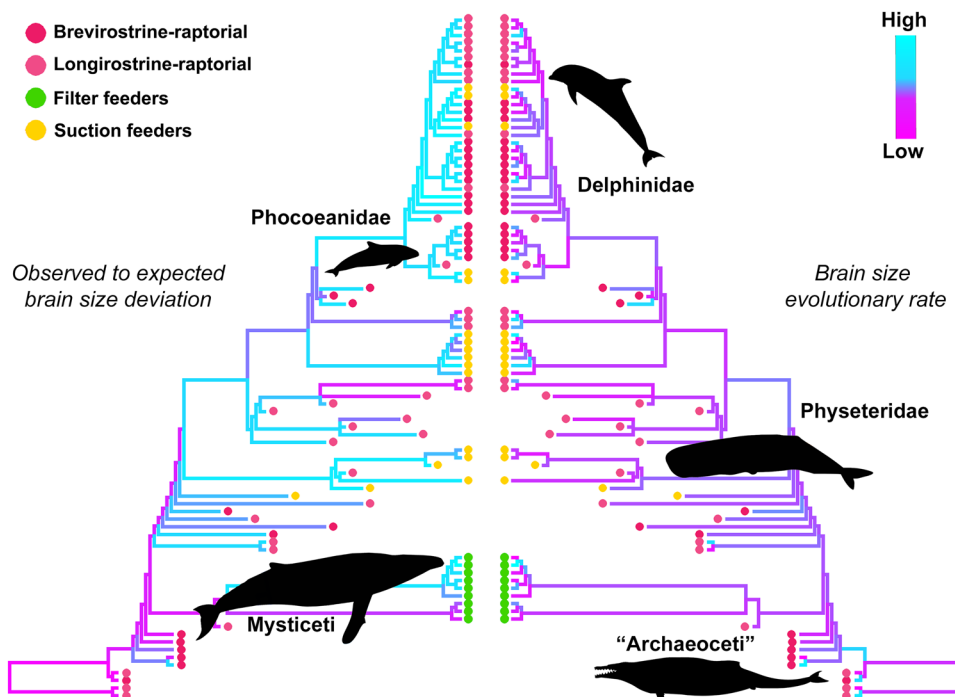
**Phylogenetic Uncertainty**

By using random trees derived from the original topology via tree swapping, we found no instance of significance for trend in body size through time, neither in Cetaceans as a whole nor in Odontoceti. Yet, we found the estimated marginal means of body size versus time regression in Odontoceti (either stem or crown) to be smaller as compared to

other species in 100% random trees (Online Resource 4, Table S7).

The regression of body size evolutionary rates through time for the entire phylogeny was significant in 2% of the random trees only. The regression slope for body size evolutionary rates in odontocetes is significantly higher than the slope of other cetaceans in 55% of the random trees when considering the crown group odontocetes. This figure

**Fig. 2** Cetacean brain size evolution along phylogenetic tree. Tree branches are colored according to the difference between observed brain sizes and the corresponding values predicted by regressing brain size versus body size (left), and to brain size evolutionary rate (right). Colored dots at tips represent the feeding categories (Color figure online)



**Table 4** (A) Estimated marginal means of brain mass versus body mass regression per feeding category and (B–C) comparison of estimated marginal means of brain mass versus body mass regression per category

(A) Estimated marginal means per group				
Suction feeders	Filter feeders	Raptorial feeders	Raptorial-brevirostrine	Raptorial -longirostrine
7.222	7.838	6.808	6.961	6.561
Estimated marginal means difference				p-value
(B) Classification by Feed3				
Filter feeders–raptorial feeders		1.030	0.720	
Filter feeders–suction feeders		0.616	0.891	
Raptorial feeders–suction feeders		–0.414	0.130	
(C) Classification by Feed4				
Brevirostrine–filter feeders		–0.877	0.909	
Brevirostrine–longirostrine		0.400	0.240	
Brevirostrine–suction feeders		–0.262	0.638	
Filter feeders–longirostrine		1.278	0.768	
Filter feeders–suction feeders		0.616	0.966	
Longirostrine–suction feeders		–0.662	0.058	

reduces to 26% when testing stem Odontoceti. The difference in estimated marginal means in body size evolutionary rates versus time regression between odontocetes and the rest of the tree is negative and significant 13% (stem) and 24% (crown) of the random trees (Online Resource 4, Table S7).

We found positive and significant phenotypic trend in brain size for the entire tree for 37 out of 100 random trees. The same figure for Odontoceti is 1% and 0% for stem and

crown groups, respectively. The estimated marginal means of brain size versus time regression for stem odontocetes are significantly higher than for the rest of the tree with 100% of the random trees. Yet, the same figure for crown Odontoceti is 2% (Online Resource 4, Table S7).

There is positive and significant trend in brain size evolutionary rates in 80% of the random trees. The estimated marginal means of brain size evolutionary rate versus time regression in Odontoceti are significantly smaller than the

rest of the tree in 93% and 95% cases, for stem and crown odontocetes respectively. The difference in brain size rates regression slopes between Odontoceti and other species is positive and significant in only 6% of the random trees for both stem and crown groups (Online Resource 4, Table S7).

The evolutionary rate shift for brain size pertaining the clade including Platanistidae and Eurhinodelphinidae was correctly identified as an instance of negative shift in rate in 78% of the random trees (Online Resource 4, Table S8). The same figure for Physterioidea is 23%, whereas we identified a positive shift in brain size evolutionary rate for Balaenopteridae and Ziphiidae in 83% and 77% of the random trees, respectively (Online Resource 4, Table S8).

## Discussion

We found evidence that Cetacean brain size increased through time. This is also true by using RBS or EQ (see Online Resource 1). Since we found no evidence for body size increase over time (Cope's rule) for Cetacea, these results indicate a genuine tendency for increased encephalization in these marine mammals (Marino et al. 2004, 2007; Shultz and Dunbar 2010; Gingerich 2015).

Montgomery et al. (2013) noted a directional trend applies to both brain and body size (but not to EQ) in cetaceans as a whole. They further noted that odontocete EQ did not follow any trend, although toothed whales have distinctly higher (and Mysticeti distinctly smaller) relative brain masses and EQs than other cetaceans. They found that by considering crown Odontoceti, these figures remain unchanged. Our results concur with theirs. We found that Odontoceti show no trend for increased encephalization through time, yet they have distinctly higher brain size than expected by their body size as compared to other cetaceans.

Although our findings comply with numerous reports attributing an outstanding encephalization grade to odontocetes (e.g. Marino et al. 2004, using EQ), our data further indicate the absence of any significant trend in the rate of brain size evolution in this clade, and negative and significant shifts in the rate pertaining to river dolphins and sperm whales. In the former we further found a significant and positive shift in body size. All of these results are consistent to sampling effects and phylogenetic uncertainty.

These results clearly indicate that the history of Odontoceti, and especially so stem Odontoceti, is characterized by high and conservative relative encephalization ever since the inception of the clade. Thus, rather than a macroevolutionary pattern, the large brain of toothed whales is best described as a plesiomorphic feature of the group.

These results are not explained by differences in feeding habits among cetaceans. In terms of evolutionary rates, raptorial longirostrine species tend to have low rates, and

FFs tend to have high rates. This suggests that longirostrine species (which mostly include delphinidae plus a number of extinct, unrelated, species such as *Albertocetus* and *Dalanistes*) started with relatively large, but conservative degrees of encephalization, that is the general pattern found among odontocetes, while Mysticeti tend to have comparatively lower but more variable brains sizes. We classified most river dolphins (*Inia*, *Pontoporia*, *Platanista*, *Lipotes*) as longirostrine (as in McCurry et al. 2017), and the FF category coincides with Chaemysticeti. Thus, the low brain size evolutionary rates in longirostrine species (which is also evident by using EQ or RBS either, see Online Resources 1, 4) is entirely coherent with the negative shifts in the rate of brain size evolution we found in platanistoid Odontoceti and probably is a phylogenetic, rather than purely dietary, pattern. This results are also consistent with Slater et al. (2010) who found early dietary diversification is associated to the buildup of cetacean morphological variation and diversification.

Prominent reasons advanced for the evolution of comparatively large brain size in odontocete cetaceans are the development of complex intraspecific interaction in their social groups (Marino et al. 2007; Shultz and Dunbar 2010) and echolocation (Marino 2004; Mortensen et al. 2014; Churchill et al. 2018). Establishing the degree of sociality for fossil species is challenging. Whereas some extinct odontocete species were reported as social (e.g. *Kentriodon*, Ichishima et al. 1994; *Dorudon*, Uhen 2004), the lack of information for most extinct taxa prevents a formal analysis of the link between social group size and brain size. Most river dolphins and orquas are indeed solitary species (May-Collado et al. 2007; Shultz and Dunbar 2010). Yet, cultural transmission via social bonds is present in some baleen whales at least (e.g. *Megaptera*, Clapham 1996; Rendell and Whitehead 2001).

Echolocation has been proposed as an explanation for the acquisition of large brain mass in Odontoceti (Marino 2004; Mortensen et al. 2014; Churchill et al. 2018). Our results partially support this notion. We found consistent evidence that Odontoceti are characterized by high and stable brain size. Importantly, the analysis of macroevolutionary rates indicate Odontoceti are more variable in terms of body rather than brain size. Echolocation appears within stem Odontoceti (the earliest toothed whale able to echolocate was the Oligocene *Cotylocara macei*, Geisler et al. 2014), suggesting echolocation might have prompted, or has at least coincided, with a consistent increase in brain size in Odontoceti (Churchill et al. 2018). This same scenario emerges by studying RBS, but not EQ. Actually, EQ and RBS represent different aspects of encephalization and possess different statistical properties. RBS is better suited to macroevolutionary investigations whereas EQ better reflects actual differences between species or groups thereof (Shultz



and Dunbar 2010). It was thus expected that macroevolutionary trends would have been more apparent with RBS, and differences between feeding categories more obvious by using EQ, as we found. However, none of the two metrics is appropriate under a phylogenetic comparative approach (Freckleton 2009). Herculano-Houzel et al. (2015) further noted they do not serve as good proxies for cognitive abilities. Although the brain anatomy of some odontocetes stands out as truly exceptional (e.g. the long-finned pilot whale, *Globicephala melas*, was noted for the extremely high density of neurons in its neocortex Mortensen et al. 2014), minke whales (genus *Balaenoptera*) possess extremely thick neocortex and a very high number of glial cells, which are known to contribute to the brain function (Perea et al. 2009). As a matter of fact, the true behavioural plasticity in large cetaceans is extremely difficult to assess (Clapham 1996) which suggests caution must be posed in interpreting the possible cause for encephalization in Odontoceti. Our results suggest that Cetacea as a whole experienced a pattern for increased encephalization, while odontocetes always have had distinctly higher relative brain mass, soon acquired at the inception of the group. The absence of a sensible trend in body size for both Cetacea as a whole and for Odontoceti indicates the slow rate of brain size evolution in the latter and the coincident evolution of extremely large body size in Chaecomysticeti is not a viable explanation for the different RBS in the two groups. Echolocation remains the most probable candidate alternative to explain the outstandingly large Odontocete brains.

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**Data Availability** All data generated or analysed during this study are included in this published article and its Supplementary Information files.

## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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