Co-evolution of cerebral and cerebellar expansion in cetaceans

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Running title: Cetacean brain evolution

Keywords: brain evolution, cerebrum, cerebellum, mysticetes, odontocetes

28   **Abstract**

29   Cetaceans possess brains that rank among the largest to have ever evolved, either in terms of

30   absolute mass or relative to body size. Cetaceans have evolved these huge brains under relatively

31   unique environmental conditions, making them a fascinating case study to investigate the constraints

32   and selection pressures that shape how brains evolve. Indeed, cetaceans have some unusual

33   neuroanatomical features, including a thin but highly folded cerebrum with low cortical neuron

34   density, as well as many structural adaptations associated with acoustic communication. Previous

35   reports also suggest that at least some cetaceans have an expanded cerebellum, a brain structure with

36   wide-ranging functions in adaptive filtering of sensory information, the control of motor actions, and

37   cognition. Here, we report that, relative to the size of the rest of the brain, both the cerebrum and

38   cerebellum are dramatically enlarged in cetaceans and show evidence of co-evolution, a pattern of

39   brain evolution that is convergent with primates. However, we also highlight several branches where

40   cortico-cerebellar co-evolution may be partially decoupled, suggesting these structures can respond

41   to independent selection pressures. Across cetaceans, we find no evidence of a simple linear

42   relationship between either cerebrum and cerebellum size and the complexity of social ecology or

43   acoustic communication, but do find evidence that their expansion may be associated with dietary

44   breadth. In addition, our results suggest that major increases in both cerebrum and cerebellum size

45   occurred early in cetacean evolution, prior to the origin of the major extant clades, and predate the

46   evolution of echolocation.

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48   **Keywords:** brain evolution, cerebrum, cerebellum, mysticetes, odontocetes

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Introduction

Cetaceans are a remarkably diverse order, varying in size from less than 40kg to 140t (Nowak, 1999; (Montgomery, 2017), but sharing a suite of derived adaptations that enable these ancestrally terrestrial mammals to occupy complex social and ecological niches in an obligatory aquatic environment. One such trait that has attracted particular attention, but remains relatively poorly understood, is a massively expanded brain. Cetaceans include species with the largest brain masses to have ever evolved (Ridgway & Hanson, 2014), and, until the emergence of the genus *Homo*, the most encephalised lineages on earth (Montgomery et al., 2013). The convergent trajectories of brain expansion in cetaceans and primates, and their possible behavioural and cognitive significance, have therefore garnered substantial interest (e.g., Marino, 1998; Marino et al., 2007).

However, differences in mammalian brain size can mask meaningful variation in brain structure and cellular composition (e.g. Barton & Harvey, 2000; Mota & Herculano-Houzel, 2014). Cetacean brains are thought to have numerous features that deviate from general mammalian trends, including a thin and highly laminated cortex, extreme gyrification, low neuron density but high synaptic density, unique neuronal cell types, and small hippocampi that lack adult neurogenesis (Breathnach, 2008; Butti et al., 2015; Eriksen & Pakkenberg, 2007; Haug, 1987; Huggenberger, 2008; Marino, 2002, 2007; Morgane et al. 1990; Oelschläger & Oelschläger, 2009; Patzke et al., 2013; Poth et al., 2005). Cetaceans also show a high degree of variation in several neural traits, including cerebellar size and cerebral cytoarchitecture (Marino, 2000; Hof & Van Der Gucht, 2007; Ridgway & Hanson, 2014; Ridgway et al., 2017, 2018).

These derived and variable neural traits make cetacean brains an informative case study in understanding the constraints acting on brain structure. Brains are structured as networks of functionally specialized, but highly integrated and interdependent, components. Their functional properties depend on both the specialised tasks of specific brain regions and their integration. Hence, the degree to which brains are able to evolve in a modular, or 'mosaic', manner has been a major, long running debate in evolutionary neurobiology. One prominent model of brain evolution argues that developmental coupling between brain structures limits the degree to which brain composition can vary, but that these constraints ensure the functional integrity of the system is maintained as brains vary in size (Finlay & Darlington, 1995; Finlay et al., 2001). This 'concerted' model is supported by apparent consistency in scaling relationships between the size of individual brain structures and total brain size across large phylogenetic distances (Finlay & Darlington, 1995; Finlay et al., 2001; Yopak et al., 2010).However, it is challenged my a more adaptationalist model in which

the development and evolution of different brain regions are at least partly independent, allowing selection to bring about adaptive changes in brain structure (Harvey & Krebs, 1990; Barton & Harvey, 2000). These adaptations are reflected by grade-shifts in the scaling relationships of specific brain regions, which indicate selective expansion that is independent of total brain size (e.g. Krebs et al., 1989; Sherry et al., 1989; Barton & Harvey, 2000; Hall et al., 2013; Barton & Venditti, 2014; Sukhum et al., 2018), and in evidence of co-evolution between functionally related structures that persist after removing the confounding effects of total brain size (Barton & Harvey, 2000; Iwaniuk et al., 2004).

While these models are not mutually exclusive, understanding the degree to which brain structure – and presumably therefore function – is limited by development is key to several evolutionary questions. In general terms, these questions are centered around how to interpret allometric scaling relationships (Huxley, 1932; Gould, 1966), and the historically important debate about the importance of developmental integration in channeling patterns of evolution (Gould & Lewontin, 1979; Arnold, 1992; Finlay et al., 1995). In the specific case of brain evolution, it is essential for understanding how behavioural specialisations are manifest in the brain, whether behavioural or cognitive adaptations are a product of whole-network properties or changes in the activity of specific operations in restricted brain regions (Logan et al., 2018), and for identifying the extent to which the genetic architecture of brain structure is the product of selection to maintain scaling relationships (Montgomery et al., 2016). Finally, given the propensity for comparisons of whole brain size when testing hypotheses about the evolution of cognition (e.g. Deaner et al., 2006; MacLean et al., 2014; Benson-Amram et al., 2016), it is critical to know whether or not these comparisons can assume relative homogeneity in brain structure across taxonomic scales, or if they are confounded by structural variance.

If mosaic changes in brain structure are common, direct comparisons of brain size can be misleading. As such, the unique morphology of cetacean brains may complicate direct comparisons with terrestrial mammals, in particular primates, where there is interest in the convergent evolution of brain expansion and cognition (Marino, 2002; Marino et al., 2007). Understanding how the differential expansion of individual brain components contributed to overall increases in brain size in each lineage is therefore crucial for accurately interpreting the significance of the convergent evolution of large brain size. One key feature of brain expansion in primates is the co-evolution and coordinated expansion of the cortico-cerebellar network (Barton & Venditti, 2014; Montgomery, 2017; Smaers et al., 2018; Smaers & Vanier, 2019; Whiting & Barton, 2003). While these structures tend to co-vary across mammals as part of a three-way relationship with the diencephalon, there appears to be a stronger co-evolutionary relationship between the cerebellum and neocortex in

126   primates (Barton & Harvey, 2000). Evidence from a range of taxa that the evolutionary trajectories
127   of components of this system can be decoupled (e.g. Hall et al., 2013; Barton & Venditti, 2014;
128   Sukhum et al., 2018), strongly suggests that the persistent correlated evolution between them reflects
129   an adaptive functional relationship.

130   In primates the expansion of cortico-cerebellar system is partly characterised by grade-shifts
131   in size, relative to the rest of the brain, that may be decoupled in time (Weaver et al., 2005; Barton &
132   Venditti, 2014; Miller et al., 2019). This implies some independent specialisation, in support of the
133   mosaic model of brain evolution, but also suggests that some form of constraint, imposed by the
134   functional integration of these structures, couples their evolution over phylogenetic timescales
135   (Barton & Harvey, 2000; Whiting & Barton 2003; Montgomery et al., 2016). Volumetrically, the
136   neocortex is the biggest component of this system, and has attracted by far the most attention from
137   cognitive and evolutionary neuroscientists (for critiques of this bias see Barton, 2012; Parvizi, 2009).
138   In contrast, the cerebellum has received much less attention, despite housing the majority of neurons
139   in the brain (Herculano-Houzel, 2009; Barton 2012). Mounting evidence suggests that the
140   cerebellum plays an important role in the development of typical and pathological variation in
141   human behaviour and cognition (e.g. reviewed in Sokolov et al., 2017), potentially through the
142   propagation of shared patterns of activity during learnt behaviour (Wagner et al., 2019), as well as in
143   the evolution of primate brain expansion and cognition (Barton, 2012; Barton & Venditti, 2014).

144   Given the accumulated evidence of cortico-cerebellar co-evolution and specialisation in
145   primates, a major question is whether or not the same pattern is observed during independent
146   episodes of brain expansion, such as cetaceans. Published comparative data on cetacean brain
147   structure has been limited but paint a complex picture of cerebellar evolution in particular. Several
148   early studies suggested that cetaceans have dramatically enlarged cerebella, with mysticetes having
149   larger cerebella compared to odontocetes as a percentage of total brain size (Breathnach, 2008;
150   Pilleri & Gihr, 1970). Marino (2000) also noted that relative cerebellum volume in two dolphins was
151   significantly larger than any primate. Several further studies have, however, noted extreme levels of
152   variation in cerebellum size across cetaceans, with some species having relatively small cerebella
153   (Maseko et al., 2012; Ridgway & Hanson, 2014). Ridgway and Hanson (2014) have also mooted an
154   apparent cetacean-specific dissociation between the normally tight correlation between the
155   cerebellum and cerebrum. Extracting general trends from this literature is therefore difficult,
156   particularly given the relatively small number of species for which data were available.

157   Recently, Ridgway et al. (2017) provided a new data set of cetacean brain structure, with
158   separate data on cerebrum and cerebellar volumes. This dataset, the result of collections made over
159   the course of 50 years, provides brain size data for 770 individuals, of which 67 have data on both

cerebrum and cerebellum volumes. These individuals unevenly represent 18 species, which makes it by far the largest dataset available to date. Using these data, Ridgway et al. (2017, 2018) presented a wide-ranging analysis of variation in brain size, structure and growth across cetaceans. Key findings include observations of highly variable brain sizes and structure between major taxonomic groups, substantial variation in cerebellar size, as a percentage of brain volume and relative to body mass, and a derived ontogeny in which prenatal brain growth is both rapid and extended (2017, 2018). Together, these results suggest that the origin and radiation of cetaceans involved substantial shifts in the selection regimes that shape brain development and structure.

However, Ridgway et al. did not compare their dataset to other mammals or examine patterns of cerebrum and cerebellum variation relative to the rest of the brain, which may be a more appropriate allometric control. They also chose to weigh individual data points equally, regardless of the number of samples per species, and to analyse their data without phylogenetic correction. Here, we revisit their data and add complementary analyses that aim to address the following questions: i) Compared to other mammals, are cetacean cerebrum and cerebellar sizes both generally expanded relative to the rest of the brain? ii) If so, do they show coordinated patterns of variation, providing evidence of cortico-cerebellar co-evolution in cetaceans? iii) Does coordinated expansion preclude independent evolution? And iv) When did these increases in size occur, and do they explain key shifts in brain size and behaviour?

Methods

Phenotypic and phylogenetic data

We obtained data on cerebral cortex (CX), cerebellar (CB), and whole brain mass from Ridgway et al. (2017) for 18 cetacean species, calculating mean masses where data for multiple individuals were available. 'Rest of brain size' (RoB) was calculated by subtracting CX and CB from total brain volume. One species, *Megaptera novaeangliae*, was subsequently excluded from the dataset as CX and CB equalled total brain mass, suggesting one or both included additional structures. Component volume data for CX, CB and RoB for an additional 124 terrestrial mammals were taken from Carlisle et al. (2017) and Stephan et al. (1981). We excluded olfactory bulbs from RoB volumes because the olfactory system is absent or greatly reduced in odontocetes (Oelschäger & Oelschäger, 2008), which, when compared to other mammals, could give the appearance of reduced RoB volumes relative to CB or CX volume. In theory, this could lead to a false signature of increased relative CB and CX size in cetaceans. The olfactory neuropil are still present mysticetes (Thewissen et al., 2011) but the available data are limited, prohibiting their exclusion in these species. However, in mysticetes the olfactory bulbs are proportionally quite small (~0.13% brain volume; Thewissen et al., 2011) so we consider their influence to have a negligible effect on our analyses. Given the small scale of deviation from isometric scaling between brain mass and volume, relative to measurement error (Isler et al., 2008), we also assume mass and volume are equivalent. Body mass was taken from the same source, with additional data from Jones et al. (2009) where data were missing. All brain and body data are available in Table S1A.

Phylogenetic trees for the included species were taken from two sources. For the analyses across mammals we use the dated supertree produced by Bininda-Emonds et al. (2007). However, the topology for cetaceans in this tree is poorly resolved. We therefore conducted cetacean-only analyses using McGowen et al.'s (2009) dated phylogeny, and spliced this tree into the mammalian supertree, re-scaling branch lengths according to the ratio of divergence dates between the last common ancestor of Whippormorpha in the two trees (Figure 1A,B). Trees were visualised using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). The spliced nexus tree is provided in the supplementary material.

Phylogenetic regressions

The core phylogenetic analyses were performed in BayesTraits (Meade & Pagel, 2016; available at www.evolution.rdg.ac.uk/BayesTraitsV3.0.1/BayesTraitsV3.0.1.html), using $\log_{10}$-transformed

224 species means. We first performed a series of phylogenetic *t*-tests using Phylogenetic Generalised
225 Least Squares (PGLS) in a Maximum Likelihood (ML) framework (Organ et al., 2007) to examine
226 variation in the size of each brain component between cetaceans and i) all available terrestrial
227 mammals, ii) terrestrial placental mammals, iii) just primates, iv) non-primate placentals. This was
228 repeated for CX and CB volume, including RoB volume as an independent variable to examine shifts
229 in relative component size. A final mammal-wide regression was performed to examine taxonomic
230 differences in CX~CB scaling. For each ML analysis, we performed 1,000 iterations and ran the
231 model with lambda, which measures phylogenetic signal, fixed to 1 and again with lambda freely
232 estimated. The fit of these models were compared using a Likelihood Ratio Test (Tables S2-5, S6).
233 We examined CX~RoB, CB~RoB, and CX~CB scaling within cetaceans using the same methods. In
234 general lambda was not significantly different from one and, where it was, it remained high.
235 However, using PMC (Boettiger et al., 2012), we found that within cetaceans our power to
236 accurately estimate lambda was reduced due to the smaller sample size, increasing uncertainty over
237 the accuracy of these model comparisons (Supplementary Information). As the results are consistent
238 regardless of whether or not lambda is estimated freely, we report the full results for both sets of
239 models in the Supplementary Information, but focus on the models with lambda fixed to 1 in the
240 main text.

241   In addition, we used phylogenetic mixed models implemented in MCMCglmm (Hadfield,
242 2010) to test whether results found within cetaceans are consistent when individual level data is used
243 rather than species means. MCMCglmm controls for phylogenetic non-independence by including a
244 covariance matrix extracted from a given phylogenetic tree as a random factor in the model. All
245 MCMCglmm analyses were performed using a Gaussian distribution with uninformative, parameter
246 expanded priors for the random effect (G: V=1,n n =1, alpha.n = 0, alpha.V= 1,000; R: V=1, n
247 =0.002) and default priors for the fixed effects. We report the posterior mean (P-mean) of the
248 cofactor included in each model and its 95% confidence intervals (CIs), and the probability that the
249 parameter value is different to 0 (P_{MCMC}).

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251 ***Rate heterogeneity***
252 We implemented the variable rates (VR) model in BayesTraits (Baker et al., 2015; Venditti et al.,
253 2011) to explore the distribution of rate heterogeneity in CX and CB evolution across the cetacean
254 phylogeny. The VR model allows the rate parameter (σ) of a Brownian motion model to vary across
255 individual branches or clades. A major advantage of this model is that it requires no *a priori*
256 hypotheses about where rate shifts occur in a phylogeny, and instead uses a Bayesian Markov chain
257 Monte Carlo reversible-jump procedure to optimise rate parameters across the tree (Baker et al.,

258   2015; Venditti et al., 2011). This is suitable for our present analyses because we are interested about

259   the presence of rate heterogeneity *per se*, and whether or not shifts in the rate of brain components

260   are co-incident, rather than in testing specific hypotheses about when or why these shifts occur.

261   We applied the VR model to CX and CB with RoB included as an independent variable in

262   each case to permit an assessment of whether there is rate heterogeneity for CX and CB evolution

263   after accounting for variation in RoB. We also performed an analysis with CX or CB included as the

264   dependent variable in models with the other component included as an independent variable to

265   confirm whether or not these traits can evolve independently. Due to the relatively small sample size

266   it is not possible to implement this model using only the cetacean dataset. The models were therefore

267   run on the full mammal dataset, and the findings therefore apply to mammals in general and are not

268   specific to cetaceans. However, evidence of rate heterogeneity within cetaceans can be inferred from

269   the branch/clade-specific scalars applied to branches within this order. The models were run for

270   100,000,000 iterations, sampling every 100,000 iterations after a burn in of 100,000,000 iterations.

271   Marginal likelihoods (MLh) were calculated using the stepping-stone sample, sampling every

272   100,000 iterations. Marginal likelihoods of the VR model were compared to the null model, in which

273   σ cannot vary across the phylogeny, by calculating a log(Bayes Factor) (BF) as:

274

275   *BF = 2[logMLh(variable rates model) – logMLh(null model)]*

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277   BFs of 5-10 indicates 'strong support' for the VR model and BFs >10 indicate 'very strong' support.

278   The VR logfile was processed using the online post processor tool (available at

279   www.evolution.reading.ac.uk/VarRatesWebPP), to extract branch lengths scaled according to their

280   mean/median rate of evolution. These were then plotted against raw branch lengths to highlight

281   periods of high CX/CB evolution (Barton & Venditti, 2014). Linear regressions between sets of

282   scaled branch lengths were performed in R (R Core Team, 2014) using the lm() function.

283   Comparisons among models were performed using Akaike Information Criterion (AIC: calculated as

284   (2 × number of parameters) - (2 × log[likelihood])) to identify the best supported model, where a

285   lower value indicates a better fitting model, and a difference between models greater than two

286   suggests a substantial difference (Burnham and Anderson 2002).

287

288   ***Ecological associations***

289   Social complexity has long been seen as a potential explanation for brain expansion in cetaceans

290   (Connor et al., 1998; Marino 2002; 2007; Marino et al., 2007), and has recently been supported by an

analysis of social repertoire size (Fox et al., 2017). As an initial test of whether social ecology is driving relative CX and/or CB expansion we obtained data on social group and repertoire size from Fox et al. (2017) and performed a PGLS regression between CX or CB with each social trait, controlling for RoB size. We also repeated these analyses using diet breadth and latitude range (also from Fox et al., 2017) as a proxy for environmental heterogeneity, maximum dive time and two tonal traits, tonal range and tonal complexity (number of inflection points). Data on dive time were taken from Marino et al. (2006), with additional and updated data from further studies (Argüelles et al., 2016; Barlow et al., 1997; Ishii et al., 2017; Krutzikowsky & Mate, 2000; Miller et al., 2010; Minamikawa et al., 2013; Silva et al., 2016). Tonal data was taken from May-Collado et al. (2007). All traits are continuous variables except for diet breadth which was coded by Fox et al. (2017) into 4 categorical groups. Data are presented in Table S1B. All analyses we performed using ML in BayesTraits with 1,000 iterations. The models were performed with lambda fixed to 1 and freely estimated (Table S6), but due to the relatively small sample size we favour the more conservative models where lambda is fixed (see Supplementary Information). All trait data has been deposited on Data Dryad (doi:10.5061/dryad.rm4368f)

Results

Both the cerebrum and cerebellum are expanded in cetaceans

All brain components are larger in cetaceans than other mammals (CX: t_{141} = 3.853, p < 0.001; CB: t_{141} = 3.814, p < 0.001), but only narrowly so for RoB (t_{141} = 2.592, p = 0.042). The scaling relationship between the CX and RoB is significantly different in cetaceans compared to other mammals (t_{141} = 6.240, p < 0.001). This is also the case between CB and RoB (t_{141} = 5.749, p < 0.001). In both cases the effect is a grade-shift toward larger component volumes than predicted by the terrestrial mammalian scaling relationship with RoB (Figure 1C,D). However, the scaling relationship between CX and CB is consistent between cetaceans and terrestrial mammals (t_{141} = 0.549, p = 0.585; Figure 1E). The same results are obtained regardless of whether cetaceans are compared to all terrestrial mammals, only placental terrestrial mammals, only primates or only non-primates (Table S2, S3).

The cerebrum and cerebellum co-evolve in cetaceans, but exceptions occur

Consistent with the comparisons between cetaceans and terrestrial mammals, within cetaceans there is a significant association between CX and CB volume after correcting for RoB volume (t_{13} = 4.453, p < 0.001). We confirmed this result, which is based on species means, using all individual-level data while controlling for species identity (P-mean = 0.653, 95% CI: 0.446-0.834, pMCMC < 0.001). We also find a potential shift in this relationship between mysticetes and odontocetes (t_{13} = -3.749, p = 0.002; Figure 2A), although the data for mysticetes is very limited (n=3) so this result should be revisited. To further explore these data we calculated the residual variance around a regression between CX volume and RoB and plotted them against the residual variance around a regression between CB volume size RoB. A non-phylogenetic regression between these phylogenetically-corrected residuals is only significant when *P. macrocephalus* is removed (present t_{15}=1.741, p = 0.102; removed t_{14}=3.238, p = 0.006; Figure 2A), after which there is again a significant shift between suborders (t_{12} = -4.596, p < 0.001). Plotting the individual data also highlights the two *Physeter* individuals as outliers to the CB~CX scaling relationship (Figure 2B). This suggests that there is a potential deviation in CB~CX scaling between mysticetes and odontocetes, and highlights specific lineages where the association between the expansion of both the CX and CB is relaxed, most notably in *P. macrocephalus* (Figure 2A). In contrast to previous studies (Ridgway et al., 2017) we do not find robust support for shifts in component scaling within odontocetes (Table S4), however, this analysis is again limited by sample size.

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Rate heterogeneity in the evolution of cerebrum and cerebellum size

We next applied a variable rates (VR) model to both CX and CB, while controlling for RoB volume, using the full mammalian dataset. In both cases the VR model was supported over a single-rate Brownian motion model (CX, BF = 25.082; CB, BF = 19.489; Table S5), providing 'very strong' evidence for significant variation in the evolutionary rate of both components that is independent of RoB volume, implying a degree of independent evolution between brain components. All variable rate models included branches within cetaceans that deviate from the background rate during mammalian evolution.

Focusing on cetaceans specifically, we plotted the *mean* scaled branch lengths against the untransformed branch lengths to visualise branches with an accelerated evolutionary rate (Figure 3A-C). The top four branches highlighted for the CB include the branch leading to the last common ancestor (LCA) of extant cetaceans, the terminal *C. commersonii* and *O. orca* branches, and the branch leading to the LCA of *B. mysticetus and E. australis* (Figure 3A,A'). For the CX the branch leading to the LCA of extant cetaceans, the terminal branches of *P. macrocephalus*, *O. orca*, and *C. commersonii* are highlighted (Figure 3B,B'). However, the more conservative *median* scalars for both components only indicate deviation for two branches for both structures, the branch leading to LCA of extant cetaceans and the terminal *C. commersonii* branch.

We next repeated the variable rates test using CX mass whilst controlling for CB volume (and vice versa, where the results obtained were highly similar, Table S5). Again, the variable rate model was supported over a single-rate Brownian motion model (BF = 28.635), suggesting that despite their tendency to co-evolve, these components have also varied independently through time. Plotting the within-cetacean *mean* scaled branch lengths for the CX and CB VR models highlights several branches with higher evolutionary rates for CX or CB (Figure 2C,C'). However, the *median* scalars only indicated deviation for the branch leading to LCA of extant cetaceans.

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Expansion of the cerebrum and cerebellum both contribute to variation in brain expansion

To explore whether increases in relative CX or CB mass drive brain expansion in cetaceans we repeated the VR analysis on brain size, while controlling for body mass, across all mammals. Again, the VR model is supported over a constant-rate model (BF = 25.467) indicating significant rate heterogeneity in the evolution of mammalian brain size when correcting for body mass. Within cetaceans, the mean scalars of each branch (indicating variation in σ) for body corrected brain size are not significantly associated with the mean scalars for either CX (t_{30} = 1.208, p = 0.237) or CB (t_{30} = 1.0885, p = 0.287) (Figure 4). However, this could reflect the dominant effect of body mass on

391   variation in relative brain size in cetaceans (Montgomery et al., 2013). Indeed, across cetaceans the

392   size of the CB (t_9 = 18.853, p < 0.001) and CX (t_9 = 98.363, p < 0.001) are significantly associated

393   with whole brain size, after accounting for RoB volume, but body mass is not   (t_9 = 2.200, p =

394   0.055). Removing body mass from the model also significantly improves the fit (ΔAIC = 3.944). We

395   take this to indicate that variation in the relative size of the CB and CX are associated with variation

396   in whole brain size. A VR analysis of brain size, without controlling for body mass, does not support

397   significant rate heterogeneity across mammals (BF = -0.326), precluding a reliable test of whether or

398   not changes in CB, CX and whole brain size occur co-incidentally in cetaceans.

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400   ***A preliminary assessment of ecological traits driving cortical and cerebellar expansion***

401   Finally, we explored the relationship between the relative size of both components and key

402   ecological variables. We first focused on social ecology, which has often been invoked to explain

403   cetacean brain expansion. We found no evidence of the predicted *positive* linear association between

404   either CX mass and either social repertoire size (t_{13} = -0.525, p = 0.608) or social group size   (t_{13} = -

405   1.734, p = 0.107), while controlling for RoB mass. This prediction is also not met for CB, where we

406   find no association between CB mass and social repertoire size (t_{13} = 0.480, p = 0.639) and a weak

407   *negative* association with social group size (t_{13} = -3.033, p = 0.010). Similar results were found when

408   CX, CB and RoB were analysed in a single multiple regression (Table S5). In the latter case there is

409   a suggestion of an association between social group size and RoB (t_{11} = 2.594, p = 0.022). We

410   repeated these analyses with whole brain and body mass and again found no significant association

411   with either social trait (Table S6).

412   Finally, we also explored the relationship between CB and CX size and diet breadth, latitude

413   range, maximum dive time, and tonal complexity (Table S6). We found only one trait with evidence

414   of an association between either brain component; both CB (t_{13} = 2.574, p = 0.023) and CX (t_{13} =

415   2.967, p = 0.011) show evidence of a positive association with diet breadth. These results are

416   however vulnerable to correction for multiple tests and should be treated as preliminary. However,

417   both associations were also present when the number of dietary categories was reduced to 3, as only

418   one species in the original dataset was assigned to category 4 (CB t_{13} = 2.484, p = 0.027; CX t_{13} =

419   2.374, p = 0.034).

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Discussion

Compared to most other extant mammals, cetaceans have evolved under dramatically different selection regimes. Comparisons between cetacean brains and those of terrestrial mammals suggest that this included changes in the selection pressures and constraints that shape how brains evolve. Using the largest available dataset on cetacean brain components, together with comparable data from terrestrial mammals, we revisited key questions about cetacean brain evolution. Despite several unique features (highlighted above), we confirm that cetacean brain expansion shares a common dependency on cortico-cerebellar expansion with terrestrial mammals, in particular primates (Barton & Harvey, 2000; Herculano-houzel & Sherwood, 2010; Whiting & Barton, 2003). Within cetaceans we find evidence of coordinated cortico-cerbellar evolution at a phylogenetic scale (Maseko et al., 2012; Montgomery, 2017; Ridgway et al., 2017; Smaers et al., 2018), but also evidence that suggests the capacity for independent changes in the size of each component. We tested three common hypotheses that seek to explain the behavioural relevance of larger cerebrums or cerebella in cetaceans, and provide preliminary evidence of a importance of diet breadth, a proxy of the diversity of prey types. Below, we discuss each of these results in further detail.

We found robust evidence that both the CB and CX are expanded in cetaceans relative to the rest of the brain, but also find a general pattern of co-evolution between them. However, this phylogenetic co-ordination appears to mask a more flexible relationship. This is indicated by significant rate heterogeneity in CX/CB volume across mammals, after accounting for their co-variation with each other or with RoB, and by individual branches showing evidence of higher rates of change in one structure or the other. We interpret this pattern as indicating a combination of distinct and shared selection pressures acting on the CX and CB, with the presence of some form of functional constraint that limits the extent to which one structure can diverge without reciprocal changes in the other (see Montgomery, et al., 2016 for further discussion). This functional dependence is consistent with known patterns of connectivity (Ramnani, 2006), coordinated activity (Wagner et al., 2019), and evidence from other mammals, particular humans, that the coordinated action of the cortico-cerebellar system is important for many behaviours (Barton, 2012; Parvizi, 2009; Sokolov et al., 2017).

An alternative explanation for the apparent co-evolution of brain components argues instead that the evolution of brain structure is constrained by shared developmental programs that couple component size to whole brain size (c.f. Finlay & Darlington, 1995; Finlay et al., 2001). One predicted pattern of a strictly concerted model of brain evolution is that structures that develop late in

457   a neurogenic time course, such as the cerebrum and cerebellum, are more prone to disproportionate

458   expansion ('late equals large' Finlay et al., 2001; Finlay & Brodsky, 2006). This hypothesis is

459   strongly debated (Weisbecker 2009), and nevertheless cannot explain our results as it argues that

460   disproportionate expansion is caused by conserved allometric scaling across groups and

461   hyperallometric scaling exponents. Our results instead provide two pieces of evidence that suggest

462   that cetacean brain structure provides a clear counter example to a general prevalence of overarching

463   developmental constraints on brain structure (c.f. Marino et al., 2000). First, major grade-shifts are

464   observed in the size of both the cerebrum and cerebellum relative to the rest of the brain; hence, their

465   increase in relative size is not due to conserved hyperallometric scaling. Second, across mammals in

466   general, and among cetaceans, there is further evidence of independent evolution of both structures.

467   Our results are therefore consistent with a 'mosaic' model of brain evolution (Barton & Harvey,

468   2000), and data from molecular studies in other vertebrates that suggest selection may act on

469   independent sets of genes and developmental pathways that control the size of each brain component

470   (e.g. Noreikiene et al., 2015; Harrison & Montgomery, 2017; Montgomery et al., 2016).

471   The question that follows, of course, is what is the behavioural relevance of these expanded

472   brain regions? Here, we focused on three hypotheses that seek to explain at least some variance in

473   overall brain size, and test whether they explain variation in either relative cerebrum or cerebellum

474   size. First, we sought to test whether variation in CB/CX size is explained by variation in social

475   ecology. The social complexity of extant cetaceans is well recognised, and includes evidence of

476   cooperative behavior, social transmission of behavior, and dynamic social structures (Connor, 2007;

477   Marino et al., 2007). Although the social complexity of odontocetes is often emphasized, many of

478   these behaviours are also observed in mysticetes (Marino, 2007; Simmonds, 2006; Whitehead,

479   2011). Several authors have suggested increases in cetacean brain size could be explained by

480   selection associated with social cognition (Connor et al., 1998; Marino, 2002; Shultz & Dunbar,

481   2010), however, evidential data has been limited. Recently Fox et al. (2017) reported an association

482   between cetacean group size, a composite measure of social repertoire size and brain size (absolute

483   and body-size corrected). We revisited these data to test whether or not group or social repertoire

484   size has a simple linear relationship with CB or CX size, independently of RoB. We found no

485   support for this hypothesis.

486   A major component of cetacean social ecology is acoustic communication. The importance of

487   auditory information arguably further increased in odontocetes following the evolution of

488   echolocation. Indeed brain structure in cetaceans has clearly evolved to support perception and

489   processing of auditory information (Marino, 2007; Marino et al., 2002; Ridgway, 2000). Cerebellar

490   expansion is also shared among mammals with pronounced auditory adaptations, including

491   echolocating bats and cetaceans, and elephants, which utilise long distance infrasonic vocalisations
492   (Hanson et al., 2013; Maseko et al., 2012; Paulin, 1993). Indeed, neural activity in the cerebellum
493   has been linked to the processing of acoustic signals (e.g. Baumann & Mattingley, 2010; Jen &
494   Schlegel, 1980; Singla, et al., 2017), and is consistent with the role of this brain structure as an
495   adaptive filter that tracks patterns of predicted and observed sensory input (Marino et al., 2002;
496   Paulin, 1993; Ridgway, 2000). We therefore next explored whether vocal repertoire (measured as
497   tonal range and tonal complexity; May-Collado et al., 2007) was associated with CB or CX mass.
498   Again, we found no significant association with either brain structure. Across social and tonal traits
499   the closest result to a nominal significance threshold of 0.05 was between RoB and group size, which
500   could suggest a potential association between social behavior and an expanded midbrain, which
501   includes several auditory structures (Marino, 2007). However, this trend was weaker for tonal traits.

502   The third hypothesis we explored is that cetacean brain composition is largely shaped by
503   foraging behavior. When discussing the striking differences between *Orcinus* and *Physeter*
504   cerebellar sizes, Ridgway and Hanson (Ridgway & Hanson, 2014) suggested that either reduced
505   visual processing or prolonged periods of oxygen depletion during deep water diving might limit
506   investment in *Physeter* cerebellar neuron number (see also Marino et al., 2006). Indeed, our analysis
507   supports the contention that *Physeter* has a unique brain composition among cetaceans, with an
508   expanded CX but relatively small CB (Figure 2). Although the data is limited, both individuals in our
509   dataset are adults, and have consistent brain compositions. Ridgway et al.'s (2017) original dataset
510   also includes two further individuals with data for CB but not CX size, which are again consistent
511   with the two individuals we include in the dataset. This suggests the small CB size observed for
512   *Physeter* is unlikely to be due to sampling biases or measurement error. However, while it is possible
513   that the constraints imposed by deep diving are particularly pronounced or limited to *Physeter*, we
514   find no general association between maximum dive time and relative CB/CX mass. Finally, Fox et
515   al. (2017) also reported an association between body-size corrected brain mass and two measures of
516   non-social ecological complexity, diet richness and geographic (latitudinal) range. While we found
517   no evidence of an association between geographic range and RoB-corrected CB or CX mass, we do
518   find a significant association between both RoB-corrected CB and CX size and a categorical measure
519   of dietary breadth. We stress that these results should be viewed as preliminary because they are
520   based on a relatively small dataset and we have performed tests for 7 ecological traits. However, they
521   are consistent with evidence that the behavioural challenges associated with foraging exert strong
522   selection pressures on the evolution of brain size and structure (Clutton-Brock & Harvey, 1980;
523   Barton 1998; DeCasien et al., 2017; Powell et al., 2017; Fox et al., 2019). We therefore encourage
524   further studies of the role of non-social cognitive specialisation in cetacean evolution.

We also acknowledge that, while we find no evidence that CB/CX expansion is driven by social ecology, our dataset (n=17) is substantially smaller than Fox et al.'s (n=46) and we do not replicate their findings with whole brain size using this subset of data. It is therefore possible that social traits do contribute to CB/CX expansion but we do not detect its effects for various reasons. First, it is possible that these null results merely reflects a combination of examining a relatively small phylogenetic dataset, and the use of behavioural data that is potentially highly "noisy", particularly given the challenge of collecting these data for cetaceans. In part, the limitations of the data come from using proxy measures of cognition. For example, Fox et al. (2017) suggest there is a non-linear relationship between group size and social complexity, and even when examining measures of social organisation (aggregations/megaopods/mid-sized associations) there is significant variation in social repertoire size, suggesting the full repertoire of social complexity is poorly captured. Similarly, May-Collado et al.'s tonal data focuses solely on tonal sounds but broadband, burst-pulsed calls also play important roles in social communication (Lammers, Au, & Herzing, 2003; Sørensen et al., 2018), and may support social interactions between individuals of species that that don't produce tonal sounds, and which do not aggregate on the surface frequently enough to accurately record social complexity (Sørensen et al., 2018). A second issue is data coverage. Despite attempts to correct for biases in publication rates (Fox et al., 2017) the availability and quality of data is likely in part determined by a species' ecology, and may not fully represent biologically relevant variation in behavioural traits across cetaceans. Even in large, comprehensive datasets, variability in trait data from alternative sources can result in differing results in comparative analyses (Powell et al., 2017), and this problem is likely to be more pronounced in hard to study species.

It is also possible that our results are influenced by different selection pressures acting on CB/CX mass in different parts of the phylogeny, or reciprocally across time. Indeed, in both cetaceans and terrestrial mammals no single ecological trait appears to explain variation in relative brain size or structure (Barton et al., 1995; DeCasien et al., 2017; Fox et al., 2017; Powell et al., 2017). In a small dataset, testing interdependencies between multiple traits is unreliable, making it hard to discern a full model of what drives the evolution of cetacean brain structure. However, given that by far the largest shift in evolutionary rate for both the CB and CX occurred on the branch leading to the last common ancestor of extant cetaceans, and that there is no pronounced shift at the origin of echolocation in odontocetes, it at least seems unlikely that CB/CX expansion was primarily driven by the evolution of echolocation, as has been previously suggested (Marino et al., 2000; Paulin, 1993; Ridgway, 2000; S. H. Ridgway & Hanson, 2014). Changes in the internal structure of the CB/CX that have been associated with echolocation (e.g. Marino et al., 2000) would therefore have evolved on the back of an already expanded cortico-cerebellar system. A similar exaptation

hypothesis has been proposed to explain how expansion of the cerebellum in apes could have initially supported increased fine motor and sequential learning needed for tool use, but was later co-opted and adapted to support the evolution of language in hominins (Barton, 2012).

An early origin of an expanded CB is consistent with some endocasts of early archaeoceti (Edinger, 1955; Kellogg, 1936 ;but see Bajpal et al., 1996; Breathnach, 1955), suggesting the switch to an obligate aquatic lifestyle may have itself altered the selection regimes acting on the size of major brain components. Indeed, there is evidence of convergent changes in cerebellar morphology between cetaceans and pinnipeds, although these are also shared by hominoid primates (Smaers et al., 2018). Teasing apart which were the key selection pressures during this period is difficult, as the shift to an aquatic environment likely involved major changes in sensory processing and motor control, both of which have been suggested as drivers of variation in CB size (e.g. Marzban et al., 2011; Maseko et al., 2012; Ridgway & Hanson, 2014). We also note that likely changes in size-related constraints on brain expansion that are associated with aquatic weightlessness, major increases in body mass (Huggenberger, 2008; Marino, 1998; Montgomery et al., 2013) and an energy-rich diet (Evans et al., 2012) may have resulted in the unique brain structure and mode of expansion characteristic of cetaceans (Marino, 2004). Although CB structure is thought to be widely conserved (Larsell, 1967; Sultan & Glickstein, 2007), the low neuronal density, non-laminar connectivity and 'cortical adjacency' of the CX (Marino, 2002; Marino, 2007) could conceivably have downstream effects on CX-CB connectivity and co-evolution in cetaceans.

Understanding the interacting selection pressures that have produced the expanded brains of cetaceans remains a daunting challenge. Given the potential for brain components to evolve independently, and to reflect complex patterns of reciprocal dependencies on other brain regions and with multiple ecological traits, we suggest that efforts to identify simple relationships between crude traits like whole brain size, and compound traits like general cognition will have limited success. Improved and more precise data for both neuroanatomical and behavioural traits is sorely needed, and the collections obtained by Ridgway et al. (2017) and others represent a major contribution towards this effort. Given the difficulty in obtaining comparative datasets, renewed long-term efforts and increased academic cooperation will be required to provide robust behavioural data, access to cetacean brain samples and imaging data, as well as tissue samples suitable for genome and transcriptome sequencing.

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Figures

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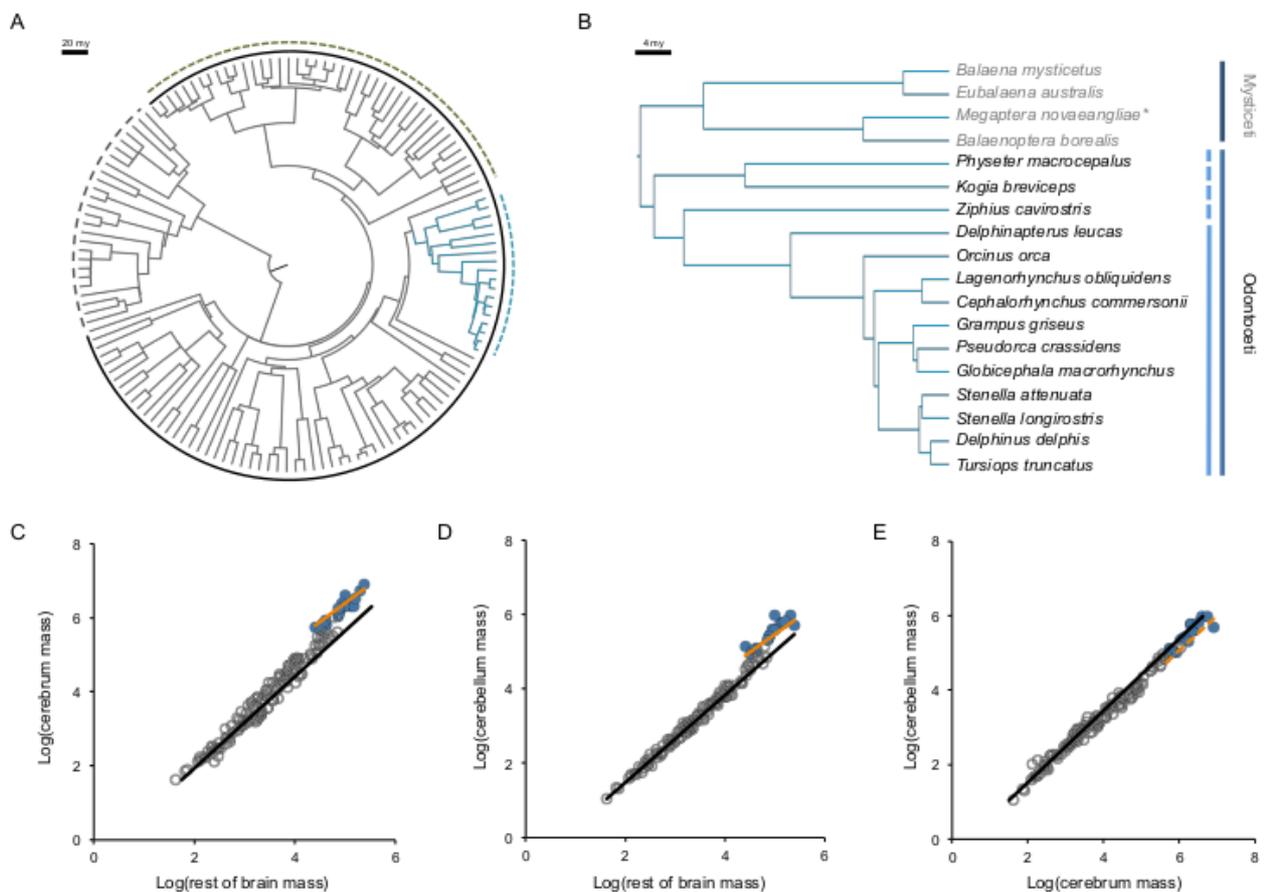
Figure 1: Phylogeny of species included in this study. **A.** The all mammal dataset. Inner dashed line shows marsupial mammals, inner solid line shows placental mammals. Outer green dashed line shows primates, outer blue dashed line and branches shows cetaceans. **B.** Cetacean phylogeny, showing major taxonomic groups. Within odontocetes the dashed/solid likes distinguish Delphinoidea from other odontocetes. Data for *Megaptera novaeangliae* (*) is available but was excluded as the sum of CB and CX equaled total brain volume, suggesting the inclusion of other components. **C-D.** Log-log plots of scaling between (**C**) CX and RoB, (**D**) CB and RoB, and (**E**) CB and CX for all mammals (grey points/black line) and cetaceans (blue points/orange line). *** indicates significant grade-shifts between cetaceans and other mammals at p<0.001, ns indicates non-significant grade-shifts.

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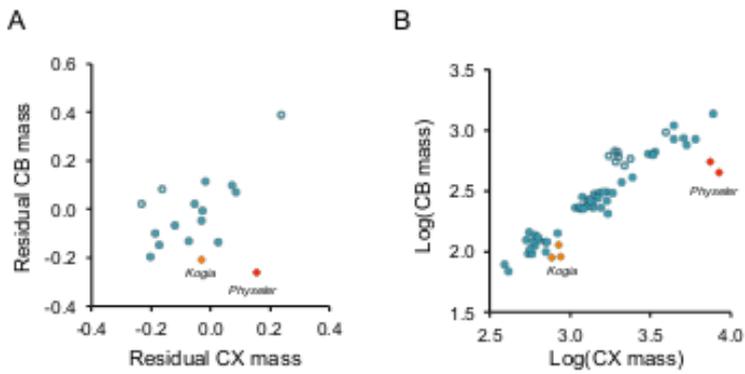
Figure 2: CB~CX co-variance in cetaceans. A. A plot of residual variance around a CB~RoB and CX~RoB regressions in cetaceans. Mysticetes are shown as in grey, odontocetes are shown in blue except for the two genera in Physeteroidea, *Kogia* and *Physeter*, which are shown as orange or red diamonds, respectively, to illustrate the position of *Physeter* as an outlier with the a smaller CB size than expected given CX/RoB size. **B.** A plot of raw individual-level data of CB~CX mass for all cetaceans, again highlighting the Physeteroidea to highlight consistency in the *Physeter* data.
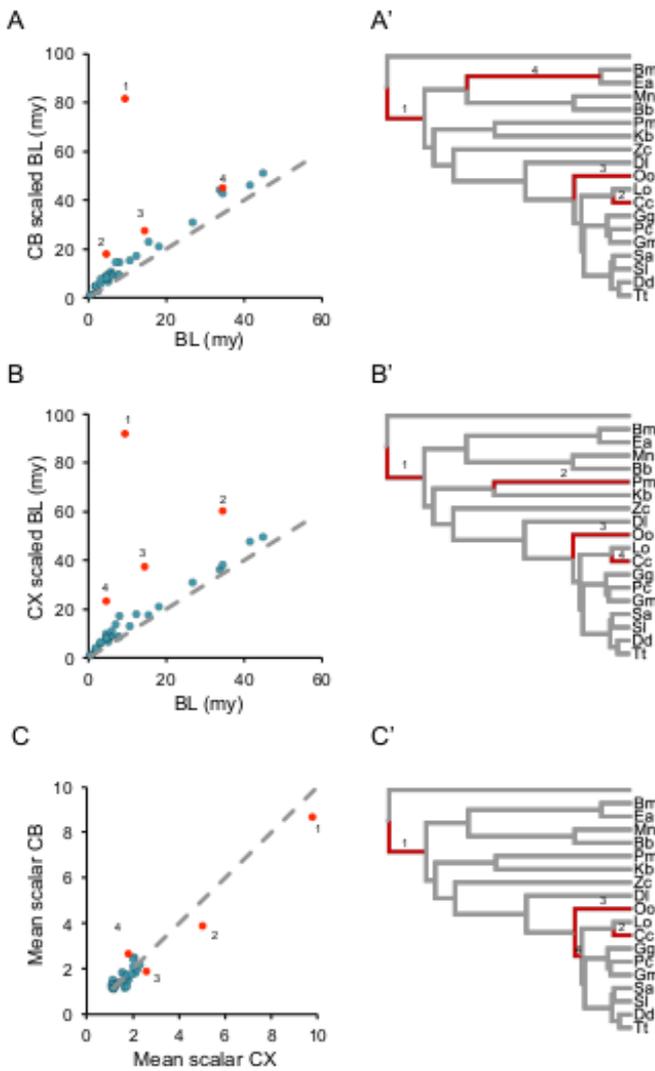
Figure 3: Scaled branch lengths from the variable rates models. A. Scaled branch lengths against untransformed branch lengths from the variable rates model for CB, numbers indicate the top four branches with the highest deviation, which are coloured red and labelled in A'. **B.** Scaled branch lengths against untransformed branch lengths from the variable rates model for the CX, numbers indicate the top four branches with the highest deviation, which are coloured red and labelled in B'. **C.** Mean scalars from the variable rates model for CB and CX, controlling for RoB, in cetaceans. Numbers indicate the top four branches with the highest deviation, which are coloured red and labelled in C'.

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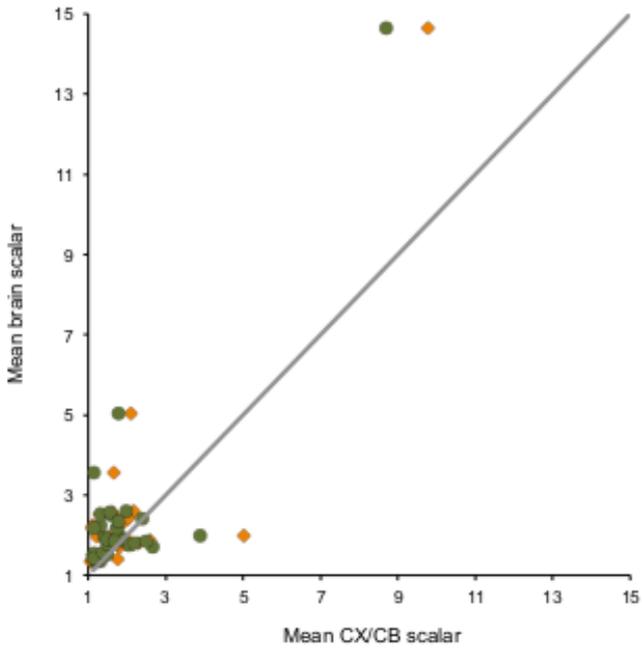
Figure 4: Mean scalars from the variable rates model for CB (orange) and CX (green), controlling for RoB, in cetaceans, plotted against the mean scalar for brain mass, controlling for body mass. The dashed line indicates a 1:1 relationship.

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Supplementary information

Table S1: Trait data

       S1A: brain component data

       S1B: ecological data

Table S3: Results of phylogenetic *t*-tests

Table S4: Results of brain scaling differences between taxa

Table S5: Results of the variable rates models

Table S6: Results of the ecological association tests

Treefile

Supplementary Information

       i) Uncertainty in estimating lambda

       ii) Power to estimate lambda

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