

The authors might consider the following in editing and improving their manuscript.

1) line 112. The authors note that, "Thus, given their pivotal role in different physiological axes, some of which have diverged extensively in cetaceans due to the conquest of the aquatic environment, it seems interesting to study their evolutionary trend in this mammalian group", but this has not been demonstrated yet in the paper, and a citation is not given for this assertion, so the text here should be adjusted. Perhaps change "some of which have diverged extensively in cetaceans" to "some of which may have diverged extensively in cetaceans"?

2) line 125. Maybe let the reader know what 'TTX' is and why this change in sensitivity is of any interest evolutionarily here? I think few people will think this is of any interest unless add more text saying why here to set up the rest of the paper.

3) Abstract - general. It might be better to frame the introduction in terms of testable hypotheses that were tested. As is, it reads as if the study is completely descriptive, which is fine, I guess. But, this might not be so compelling to a general reading audience.

4) line 131. It would have been of interest to sample a hippopotamid as these species are semi-aquatic and the extant sister group to Cetacea and have decent genome assemblies I think. Was there a reason that these were not sampled? Would it be possible to include these, or would that require doing everything over from the start?

5) line 140. I do not know if this is the best approach to pulling out these genes. Has such an approach been used in other studies (or an analogous approach), or is the sequence of steps in this paragraph novel to this study. It might be good to perhaps justify each step a bit more, or the overall approach, to convince the reader that this is a decent pipeline for pulling out the desired set of coding sequences for ion channel genes from the genomes examined here.

6) line 166. In this section, it should be noted whether dN/dS analyses were done in which different dN/dS was permitted on the stem and/or crown Cetacea branches. If not done, why not? It would seem that it would be good to test for significant shifts in selection intensity at the transition to aquatic environment and also within the crown Cetacea lineages which all represent evolution in obligately aquatic mammals, in contrast to the outgroups (terrestrial) and the stem Cetacea branch (transition to fully aquatic). Here again, I think it would be good to include one or both extant hippos in the analyses, since these are the closest extant relatives of Cetacea. For the models described in this section, it seems that what will be inferred is positive selection in a subset of sites, or not. But, is that the best or most interesting question?

7) line 181. The breakdown of branches here might be useful to try for the dN/dS analyses (e.g., separating Cetacea from other mammals). However, note that 'stem Cetacea' as delimited in the current study includes also stem Cetancodonta. Because hippos are not included, some of this 'stem Cetacea' branch includes evolutionary history that is prior to the divergence of Cetacea from Hippopotamidae. As noted above, I think it would be useful to include hippo genomes in this study, for a variety of reasons.

8) line 189. Clarify what 'adjusted' means here, presumably some sort of correction for multiple tests (or some other)?

9) line 216. How much smaller? Is this a problem? Also, for Delphinidae, *Tursiops* is maybe not as complete a genome assembly as *Orcinus* (?). Was genome quality correlated with number of genes pulled out of these genomes? Using more species that are closely related might have helped to assess the effects of varying genome quality on the numbers of these genes in different genomes.

10) line 220. The "unpaired one-tailed t-test" is not appropriate when comparing different genomes within a phylogenetically coherent way as, for example, the different genomes within Cetacea are not independent data points due to shared common ancestry to varying degrees, so some other test should be utilized here (i.e., one that takes phylogenetic structure into account).

11) line 221. I think the statement "This result is consistent with the hypothesis that gene loss can play a significant role in phenotypic evolution" needs more explanation here. All mammalian taxa analyzed here have unique traits and differ greatly in phenotype. For example, if there were a huge reduction in gene number in human, would this also be consistent with "the hypothesis that gene loss can play a significant role in phenotypic evolution"? Humans are highly derived, large brained primates that walk on two legs and have complex societies. At any rate, I think the statement here is fairly unconvincing; if cetaceans had way more gene copies than other taxa, would the exact same statement be made, or if highly derived flying bats had fewer copies (which is the case), etc., etc. In part, this relates back to the question regarding having prior hypotheses at the start of the study, rather than sort of just describing/documenting things and having to then consider plausible explanations as you go along.

12) line 252 and following paragraphs. Are the positive selection signals for 'heart genes' on the cetacean lineages or across the whole tree? If there is no specific evidence of positive selection just on the cetacean 'stem lineage' and in crown Cetacea, why infer that that adaptation in cetaceans is driving the high dN/dS in these genes. According to the methods, it does not appear that cetacean and 'background' branches (non-cetacean branches) were partitioned such that different dN/dS are permitted for these different categories. Unless I am not understanding something, I do not see how the authors can make the inferences they are trying to make given the results that they have presented.

13) line 299 and following paragraphs. This section is quite speculative and rambling. Why is the mutation not in mysticetes? There is a further reversal in *Tursiops* with a speculative explanation for that as well. As the authors note, all of this needs to be tested experimentally, and I am not sure that the amount of text here is warranted given the speculative nature of all of this. But this is potentially interesting.

14) line 447. I have worked on cetaceans for over 30 years, I am not convinced that "Hearing is undoubtedly the most critical sense for life underwater", and I am not sure that this statement is even true, no less "undoubtedly" true. I would go with sight probably, and the importance of sight vs. hearing varies considerably among different lineages of cetaceans that are specialized in different ways.

15) lines 579-581. I do not think this statement is supported by the results of the analysis. This is possible, of course, but is a leap in logic certainly.