Dear Gavin, here, you will find a revised version of our manuscript incorporating all the changes suggested by the reviewers. We also thank you for the time you invested in providing constructive criticism. The most significant change is that we ran all analyses from scratch, including the hippopotamus and two other cetacean species (Vaquita and Narwhal). The new results remain the general trend we reported in our first submission. We hope you consider that the revised version is now in good shape to be recommended in PCI genomics.

I agree with Reviewer 1's point that the authors have not provided clear motivation for why they have chosen to study ion channels. Further details should be given to explain (and justify) the claim that physiological axes have diverged in cetaceans.

The reviewer correctly points out that we need to support this statement. To solve this problem, we provide references at the end of the statement. Now, the new statement reads as follows: "*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 (Varró et al. 2021; Poole, 2022; Kashio & Tominaga, 2022)"*

Please provide justification for why so few non-cetaceans were included (given that there are many more mammals that could have been included). I think it would be much more convincing that cetaceans were major outliers genomically in mammals if a more diverse set was compared to. This is highly related to Reviewer 1's point 11.

We understand this concern; the first reviewer also raised that. To deal with this problem, we added three more species, two cetaceans (vaquita and narwhal) and the hippopotamus, and we ran all analyses from scratch. The inclusion of the hippopotamus was the most important one, as highlighted by the reviewer. Although our study is in better shape after expanding our taxonomic sampling, it is interesting that the main results stayed the same. Further, some of our results agree with other previously published studies, making us feel confident about our results.

You report that there are significantly fewer ion channel genes in cetaceans vs. non-cetaceans (which is true, based on a t-test at least), but actually there appears to be a stronger signal of fewer protein-coding genes in general in cetaceans vs. non-cetaceans, which seems more relevant.

We appreciate this comment. The reviewer is correct; the comparison of the number of protein-coding genes between cetaceans and non-cetacean mammals indicates that the first group possesses fewer protein-coding genes (Unpaired one-tailed t-test: t = -4.9399, df = 14.886, p-value = 9.097e-05). To highlight this issue, we included a paragraph that reads as follows: *"Interestingly, we found that cetaceans possess fewer protein-coding genes than non-cetacean mammals (18845.5 ± 977.29 vs. 21396.22 ± 1218.75, unpaired one-tailed t-test with d.f.=14.88; t-statistic = -4.94 and p-value = 9.1e-5). This result is consistent with other studies in which a reduction in gene copy number in cetaceans and other groups is associated with evolutionary innovations (Feng et al. 2014; Nery et al. 2014; Sun et al. 2017; Huelsmann et al. 2019; Helsen et al. 2020a; McGowen et al. 2020; Cabrera et al. 2021; Randall et al. 2022; Zheng et al. 2022; Osipova et al. 2023; Pinto et al. 2023)."*

The actual percentage of ion channels does not appear lower (and could actually be higher), as displayed in Figure 1. This suggests that many gene groupings are likely at lower absolute copy

numbers, and not ion channels specifically. Is this true (e.g., if you look at genes grouped based on different protein 2 domains)? Or are ion channels specifically depleted? The current description of the results would be misleading unless the latter is true.

We appreciate this criticism. The comparison of the proportion of ion channels between cetaceans and non-cetacean mammals indicates that the first group possesses more ion channels than terrestrial mammals (Unpaired one-tailed t-test: t = 2.933, df = 13.587, p-value = 0.0056). ***To solve this problem, we introduced a paragraph describing this result. The new text reads as follows:*** **"*On average, cetaceans possess a higher proportion of annotated ion channels in their genomes than the non-cetacean mammals (9.95 ± 0.38 vs. 0.92 ± 0.61, unpaired one-tailed t-test with d.f.=13.587; t-statistic = 2.933 and p-value = 0.005). Although the literature contains abundant examples of gene loss reported for cetaceans (see references above), there are also examples in which cetaceans expanded their gene repertoire. For instance, Holthaus et al. (2021) report that a subtype of small proline-rich proteins has expanded in copy numbers in cetaceans. Genes related to tumor suppression, cell cycle checkpoint, cell signaling, and proliferation have also expanded their repertoire in cetaceans (Tollis et al. 2019; Tejada-Martinez et al. 2021).*"**

In addition, I think showing the distributions of the gene counts of all protein-coding genes and for ion channels in the two lineages separately would help readers pick up that the overall numbers of genes are lower (although using a phylogeny-informed statistical test, as suggested by Reviewer 1, would be good).

Thank you for this insightful suggestion. It led us to chart the distributions of gene counts for all protein-coding genes and ion channels within each lineage separately. This analysis revealed contrasting averages between the lineages, particularly in terms of the absolute frequency of ion channels versus their proportion among protein-coding genes.
Given the sample size, we opted to present the distributions graphically using Kernel Density Estimation. These functions transform discrete data into continuous curves, allowing for a smoother distribution visualization. Additionally, we color-coded the curves according to the respective lineages to facilitate interpretation. We appreciate your valuable input, which has enriched the analysis and presentation of our results.

Starting at L242 you describe what functional categories genes displaying signals of positive selection are enriched for. A summary of the signals of positive selection identified is first needed. For instance, how many genes out of how many tested were significant? What were the effect sizes? How did the results differ based on the two sets of models compared? It would also be good to remind the reader what the general analysis was (e.g., that it was restricted to ion channel genes).

We agree with this comment. In the revised version of the manuscript, we included a small paragraph showing the requested information.

The authors claim that their positive selection results "emphasize the importance of ion channel genes in adapting to diving" (L261-262), but this is not convincing as they only scanned for signatures of positive selection in ion channel genes. It is very possible that many kinds of genes show evidence of positive selection, and that ion channel genes are not especially enriched for this signal compared to the entire genome. The authors would need (1) to compare to other gene categories to convincingly show that ion channel genes in particular display evidence for positive selection, and (2) show that this signal is restricted to cetaceans rather than mammals in general.

We agree with this comment. When we say, "Our findings emphasize the importance of ion channel genes in adapting to diving," we mean that they are important but no more important than others. To solve this problem, we reworded this sentence. The new text reads: "*Although the basic structure of the cetacean heart is similar to that of other mammals, our findings emphasize the importance of ion channel, among other proteins, in adapting to diving.*"

Related points:

My point #2 above is very similar to Reviewer 1's point 12: if the authors are making claims about higher levels of positive selection specifically in cetaceans, then this must be relative to other mammals, but based on the methods I do not believe that non-cetaceans were tested for signatures of positive selection.

The reviewer is right, we used site analyses, something that in the revised version is emphasize.

What was the background set of genes used for the Enrichr analysis? Based on the methods it sounds like only ion channel genes were tested for positive selection.

The reviewer is correct, we only analyze orthologous groups containing ion channels.

Table 1 only a small number of unique genes are listed as associated, so this suggests that the signal of positive selection could be restricted to just a few ion channel genes (if there are similarly small gene sets for the other phenotypes discussed in addition to heart physiology).

The reviewer is correct. The mammalian phenotype ontology database shows the genes associated with each recognized category and could be repeated in different categories. For example, there are 18 genes related to heart physiology, where some genes appear once (e.g., RYR3), while others are more frequently mentioned (e.g., SCN5A). In any case, we condensed the tables in one figure in the revised version of the manuscript. The tables are now supplementary material.

Clarification is needed that many of your results are bioinformatic predictions of phenotypes rather than actual observations of phenotypic differences. Two (non-exhaustive) examples are listed below.

L55 – 'seems to be sensitive to TTX' implies observed sensitivity (or at least could be interpreted that way). This sentence should be re-worded to clarify that you predict sensitivity.

The reviewer is correct, to solve this problem we changed the wording according to the suggestion. The new sentence reads: "*Interestingly, we predict that the NaV1.5 ion channel of most toothed whales (odontocetes) is sensitive to TTX, similar to NaV1.7, given the presence of tyrosine instead of cysteine, in a specific position of the ion channel.*"

L262 – 'adapting to diving' should be clarified that this is only a possible link. This is a hypothesis and that there is no direct evidence of a link between the elevated dN/dS in some genes and adaptation to diving.

The reviewer is correct, to solve this problem we changed the wording according to the suggestion. The new sentence reads: "…….*our findings hypothesized the importance of ion channels, among other proteins, in adapting to diving*"

I agree with both reviewers that the section describing the scanning of human-pathogenic variants in cetacean ion channel genes is overly speculative. This information could be useful but there is very little evidence to support the speculations. Human-pathogenic variants could have entirely different effects in the different genetic and environmental background of cetaceans, so I do not think much can be concluded from this analysis. In addition, for at least some of the analyses (e.g., Table 2) the authors do not provide information on the distribution of these variants in non-cetaceans beyond humans, meaning that it is possible that the non-human-pathological state is recently derived, and the pathological state in humans is ancestral.

We agree with this comment, to solve this problem we removed all the information regarding the human pathogenic variants.

Formatting comments
I could not find the link to your Zenodo repository within your preprint itself (although I could find it through the PCI Genomics portal). Please make sure this is included in a separate section titled "Data, script, code, and supplementary information availability".

Done

I appreciate that you provided a Word document accompanying your scripts that describes your exact bioinformatic steps, but I strongly suggest this be changed to plain text format as sometimes special characters can cause issues when commands are copied and pasted from Word.

Done

Please move your funding information from the acknowledgements to a separate subsection called "Funding".

Done

Also include a separate section called "Conflict of Interest disclosure" indicating any conflicts or confirming that you have none.

Done

Please use a consistent reference style in your reference section. Note that many journals, including Peer Community Journal, request that DOIs be included in the reference list.

We used the program paperpile to format our references, using the Genome Biology and Evolution style.

Minor comments
L53: Should be "a signal of positive selection", not "the signal".

Done

L56: Please write TTX out as tetrodotoxin in the abstract (and likely this would be better written out in the keywords as well).

Done

L78: I suggest ‘Thus,’ be removed (or replaced with ‘Indeed,’), as this sentence is not a necessary consequence of the preceding point.

Done

L82: Should re-word ‘translates their solution to us’ to be something like ‘, from which we can potentially gain medical insights’.

Done

L107: I would re-word ‘ion channels have been estimated’ to ‘putative ion channels have been identified’.

Done

L147: Capitalize ‘E-value’.

Done

L149: It would be good to be more explicit by what you mean by ‘We then compared’. Figure 1 implies that you limited hits to those that intersected both, but it would be good to be clear about that in the text.

We agree with this comment. To solve this issue, we reworded this section of the manuscript. The new text reads: "*To identify the ion channels from our list of proteins, we prepared a file containing the list of ion channel conserved domains based on the Conserved Domain Database (CDD) (Lu et al. 2020). Having done that, we intersected it with the results from RPS-BLAST v2.13.0+ followed by rpsbproc. This was done using an in-house Perl script to identify the ion channel repertoire for all sampled species (Fig. 1).*"

L170-171: Please briefly explain the difference between the two sets of models that are compared (i.e., explain why there isn’t just one set of nested models compared).

We appreciate this comment. Lines 171-172 already contain a brief explanation. The PAML program is well-known, and most scientists are familiar with it. Even if further details are required, the manual is well-written and contains all the information.

L172: ‘null model (M1a and M7)’ should be ‘null models (M1a and M7), which’.

Done

L181: ‘other’ should be ‘another’.

Done

L216: I suggest the comma after ‘literature’ be changed to a colon.

Done

L227-229: The orthologous groups are gene families encoded by specified species, so they are present in the species' genomes rather than the species present in the groups. This should be re-worded to reflect this distinction.

We apologize, but we do not understand the proposed change. In any case, we consider how we present the information will be understandable for most readers. We think that the term orthologous group is well recognized and understood in the scientific community.

L247-249: Should clarify what you mean by 'studies where groups of genes related to specific characteristics are studied', as it's not clear whether you are referring to the same actual overlapping functions/genes, or just similar functions, or whether you just mean any genotype/phenotype comparison more generally.

We agree with this comment. To solve this problem, we replaced "characteristics" with "phenotypes."

L299-301: Should specify that these known polymorphisms and observations have been in humans.

All this information was removed.

L447-448: I suggest you remove 'In fact, the cetacean hearing has evolved to be remarkable' (while clarifying this section as suggested by Reviewer 1).

That sentence was modified according to reviewer 1.

L524: 'fasta' should be 'FASTA'.

Done

Reviewer #1
lines 149 and 747. Change reference from 2020 by the updated one from 2023. https://pubmed.ncbi.nlm.nih.gov/36477806/ PMID: 36477806

Done

The authors might consider the following in editing and improving their manuscript.
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 some of which have diverged extensively in cetaceans" to "some of which may have diverged extensively in cetaceans"?

The reviewer correctly points out that we need to support this statement. To solve this problem, we provide references at the end of the statement. Now, the new statement reads as follows: "*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 (Varró et al. 2021; Poole, 2022; Kashio & Tominaga, 2022)"

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.

We understand the reviewer's concern. To solve this problem, we reworded this text, now it reads as follows: "*3) the $Na_V1.5$ ion channel of toothed whales (odontocetes), other than species of the genus Tursiops, is predicted to be sensitive to the potent neurotoxin tetrodotoxin (TTX), similar to $Na_V1.7$, given a replacement of cysteine for a tyrosine*"

The reader will see further details in the 1.5 pages we devoted to this discovery (lines 328 to 373).

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.

We understand the reviewer´s concern but do not see a problem presenting a descriptive scientific work. In our way of thinking, descriptive studies also have a fundamental role in advancing science (Grimaldi & Engel 2007; Casadevall & Fang, 2008).

Grimaldi & Engel. 2007. BioScience (https://doi.org/10.1641/B570802)
Casadevall & Fang. 2008. Infection and Immunity (https://doi.org/10.1128/iai.00743-08)

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?

The reviewer is correct. The new version of our manuscript incorporates the hippopotamus and two other cetaceans.

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.

We downloaded the protein-coding sequences from the world's largest public resources of biological sequence databases, with a long-term tradition (over 20 years, CDD, Wang et al. 2023) of genetic data curation and storage (https://www.insdc.org/). Further, figure 1 provides a graphical explanation of our bioinformatic pipeline with a reasonable way of detail. If the readers have questions regarding the databases used in our publication, they can check the papers we cite (Yates et al. 2022; Sayers et al. 2022).

Yates AD, et al. 2022. *Nucleic Acids Res.* (https://doi.org/10.1093/nar/gkac1096)
Sayers EW, et al. 2022. *Nucleic Acids Res. (https://doi.org/10.1093/nar/gkab1112)*
Wang J, *et al.* 2023. *Nucleic Acids Res. (https://doi.org/10.1093/nar/gkac1096)*

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 envirnment 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?

We understand the reviewer's concern. Although the most obvious way of thinking is to test the stem and the crown, expecting most changes to occur in the stem, this pattern is only sometimes true. This also holds for other forms of genetic variability, not only dn/ds. For example, according to our results, the highest value of gene turnover rate was estimated for the crown group cetacea. Further, the value estimated for the stem was four times lower than for the non-cetacean species included in our sampling. In the case of dn/ds, although we did not report results, we also ran branch models, and in all of the instances in which we estimated separate omega values for the crown and the stem, the crown value was higher. We also ran branch-site tests labeling the stem cetacea as the foreground branch, not obtaining any gene with the signature of positive selection. In our way of thinking, all these results suggest that most of the "*evolutionary activity*" is happening in the crown group. For this reason, we ran site analyses, which are used to identify positively selected sites in a multiple sequence alignment in the group of interest. The statistical power of site-specific models has been demonstrated in the literature (Anisimova et al. 2001; Yang & Bielawski, 2000; Yang & Nielsen 2002). Interestingly, these results, i.e., that most of the "*evolutionary activity*" occurred in the crown group cetacea, were very similar when we studied the evolution of tumor suppressor genes (Tejada et al. 2021).

Anisimova et al. 2001. Molecular Biology and Evolution. https://doi.org/10.1093/oxfordjournals.molbev.a003945
Tejada et al. 2021. Proceedings of the Royal Society B. https://doi.org/10.1098/rspb.2020.2592
Yang & Nielsen. 2002. Molecular Biology and Evolution. https://doi.org/10.1093/oxfordjournals.molbev.a004148
Yang & Bielawski. 2000. Trends in Ecology and Evolution. https://doi.org/10.1016/S0169-5347(00)01994-7

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.

The reviewer is correct. To solve this problem, we ran all the analyses from scratch, including the hippo and two other cetacean species (Narwhal and Vaquita).

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

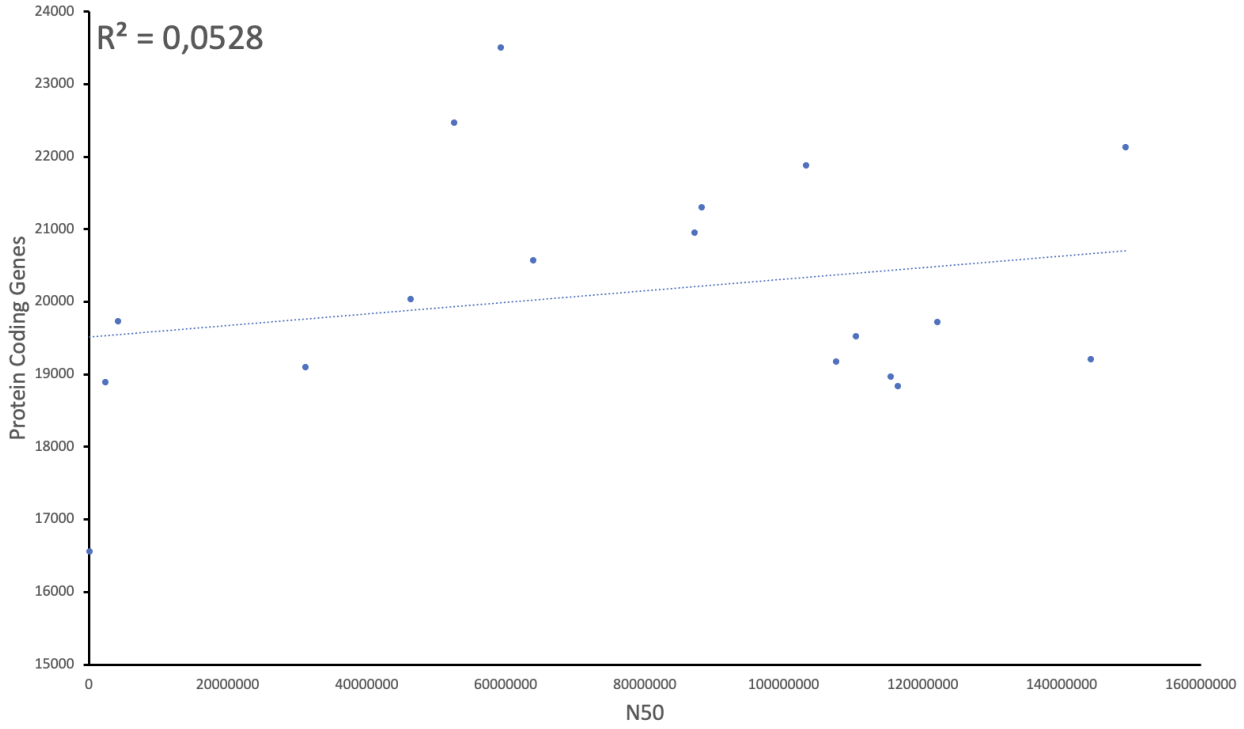
Thank you for noticing this. We have added an explanatory sentence with the original reference from Benjamini & Hochberg (1995). The new text reads as follows: "*The adjusted probability is calculated from the resulting list of categories with raw p-values equal or lower than 0.05, through the procedure of False Discovery Rate (chosen FDR is also 0.01)*

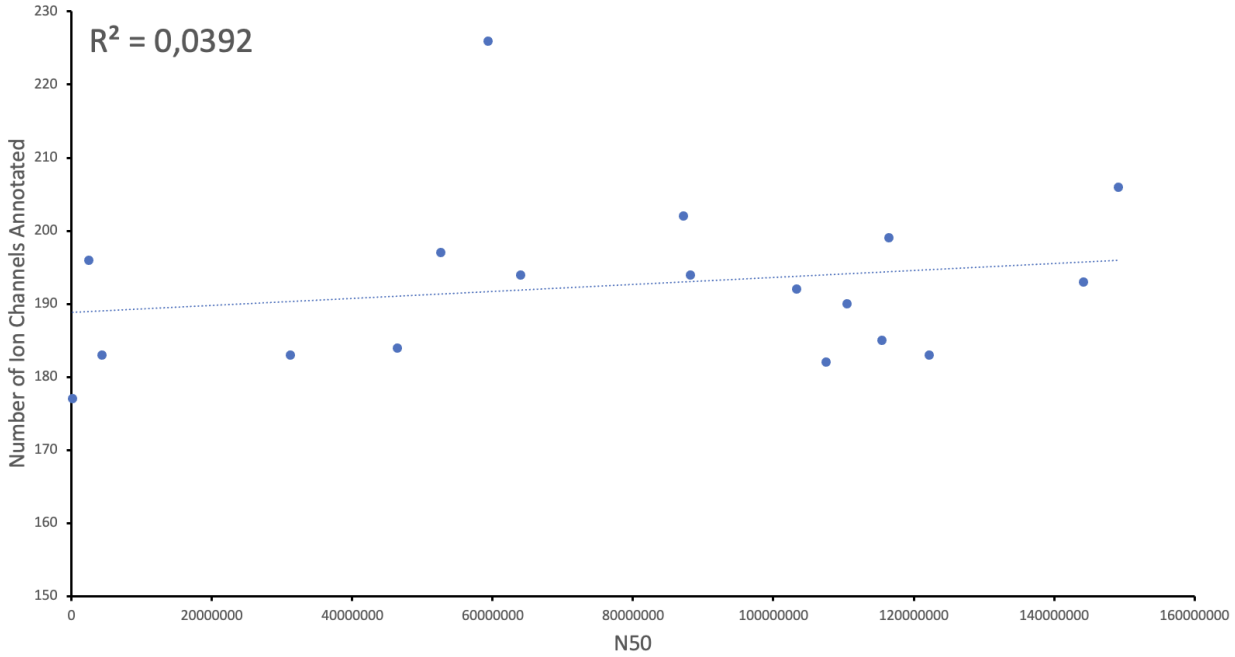
***(PMID). The aim of FDR is to reduce the final number of false positive categories in the results.*"**

Benjamini & Hochberg. 1995. Journal of the Royal Statistical Society: https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

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.

In the manuscript, we reported the values obtained according to our bioinformatic pipeline (human, 226; mouse,197) and the values reported in the literature (human, 235; mouse, 231). The difference is not a problem; it is just an update since the last estimation occurred 14 years ago. To avoid confusion, we modified the statement, and now it reads as follows: "*Our results are comparable to what is reported in the literature, 235 ion channels for humans (Homo sapiens) and 231 for the mouse (Mus musculus) (Jegla et al. 2009).*"
Regarding a possible correlation between genome quality and the number of protein-coding genes or annotated ion channels, we found no correlation (0.229 Pearson, p-value = 0.35, and 0.198 Pearson, p-value = 0.4707, respectively).





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).

We agree with this comment. Although we reported this phylogeny-independent test in our manuscript, we also performed a test in which the phylogenetic relationships and divergence times of the species included in our sampling are considered, i.e., gene turnover rate estimation using the software CAFE.

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 uncompelling; 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.

We understand the reviewer's argument, and it is possible to fix this problem by rewriting this passage, stating that our result agrees with other studies in which fewer gene copies have been reported as a source of phenotypic innovation. The new passage reads: "*This result is consistent with other studies in which a reduction in gene copy number in cetaceans, and other taxonomic groups, are associated with evolutionary innovations (Feng et al. 2014; Nery et al. 2014; Sun et al. 2017; Huelsmann et al. 2019; Helsen et al.*

***2020; McGowen et al. 2020; Randall et al. 2022; Zheng et al. 2022; Osipova et al. 2023; Pinto et al. 2023).*"**

In addition, we deleted the following statement: "*This result is consistent with the hypothesis that gene loss can play a significant role in phenotypic evolution (Olson 1999; Albalat and Cañestro 2016; Helsen et al. 2020).”*

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.

The confusion comes from the misunderstanding regarding the site analyses. This type of analysis, used to identify positively selected sites in a multiple sequence alignment in the group of interest, includes only sequences (and phylogenetic relationships) from cetaceans. This is why we can generalize for the cetacean group. To avoid misunderstandings, we added the names of the models we used.

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.

We understand the reviewer's concern. It is not possible to answer the question of why the mutation is not present in mysticetes. We can show when the mutation occurred based on how the species in our sampling are related. A similar situation occurs regarding the reversal in the ancestor of the genus *Tursiops*. As we mentioned in the manuscript and noticed by the reviewer, the ideal would be to test the protein with the mutation experimentally. However, in this case, we feel lucky as, in the literature, the sensitivity for TTX has been extensively studied. The main conclusion is that "*this residue is the structural determinant that differentiates the TTX-insensitive sodium channels (Nav1.5 and Nav1.8–Nav9) with a Cys or Ser from the TTX-sensitive channels (Nav1.1–Nav1.4, Nav1.6, and Nav1.7) with a Tyr or Phe*" (Jiang et al. 2020). So, based on the argument exposed, we feel that our writing is not very speculative.

Jian et al. 2020. Cell. 180: 122. (https://doi.org/10.1016/j.cell.2019.11.041)

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 "undoubtably" 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.

We understand the reviewer's concern. Based on the new analyses, including more species, the hearing was not among the top categories, so this text was removed.

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.

We understand the reviewer's concern. To fix this problem, we removed that statement.

Reviewer #2

This study employs a bioinformatics pipeline to investigate the evolutionary dynamics of ion channels in cetaceans. The findings reveal a reduction in the repertoire of ion channels in cetaceans compared to their terrestrial mammalian counterparts. Notably, the NaV1.5 ion channel in most toothed whales exhibits specific amino acid variations deemed pathological in humans. Particularly, a significant proportion of these whales possess a tyrosine residue at a precise position within the NaV1.5 channel, potentially rendering them more susceptible to certain toxins. These discoveries offer profound insights into the mechanisms underpinning cetacean adaptations to their aquatic habitat. This research not only presents intriguing implications but also holds substantial scientific significance. The study encompasses a variety of functionalities related to ion channels, including cardiac and skeletal muscle contraction, echolocation, and polycystic kidney syndrome. However, experimental validation of these bioinformatic analyzes is necessary and requires in-depth investigation of the specific functions of ion channels.

We appreciate all the positive comments. We agree that experimental validation is necessary for further understanding the genomic bases of the conquest of the aquatic way of life of cetaceans. However, it is out of the scope of our work. In the future, scientists who do experiments will take some of our results to the bench.