

Monday 11 August 2025

To whom it may concern,

The PhD thesis by Valeriia Telizhenko, entitled "Genetic and Morphological Proxies for Evolutionary Changes in the Ontogeny of Cetacean Skeleton," presents an ambitious and comprehensive investigation into the evolutionary developmental biology of cetaceans, integrating morphological, molecular, and phylogenetic approaches to understand the genetic and developmental foundations of key cetacean skeletal adaptations. Conducted at the I.I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, the work addresses fundamental questions about how major evolutionary transitions are achieved through modifications of developmental programs, focusing on two distinctive aspects of cetacean biology: delayed cranial ossification with associated accessory bone formation, and the evolution of limb development genes underlying forelimb morphological diversity. The thesis demonstrates substantial scholarly merit, as evidenced by publication of three core chapters in high-quality international journals (*Scientific Reports*, *Communications Biology*, and *Proceedings of the Royal Society B*), along with presentations of findings at major international conferences. Ms. Telizhenko's multidisciplinary approach represents a significant contribution to our understanding of the developmental mechanisms underlying one of the most remarkable evolutionary transitions in mammalian history.

Chapter-by-chapter assessment – This review walks through each chapter and includes technical as well as broader comments for ease of reading.

Chapter 1: Chapter 1 provides a comprehensive literature review providing essential background information for understanding evolution and development of cetaceans. The uniqueness of the cetacean body plan due to a fully aquatic lifestyle is outlined, and potential drivers for these changes from a terrestrial body plan are outlined. Generally, it is a thorough review, but it might be worth reviewing and discussing the results of Coombs et al. 2020 and Lanzetti et al. 2022 in the section discussing asymmetry of the skull. The Lanzetti paper is mentioned, but the Coombs et al. paper seems to strongly support that skull asymmetry is associated with toothed whale echolocation abilities. This could be addressed with perhaps a sentence or two. Additionally, there are some organizational issues in the physiological adaptations section. On page 29, a paragraph begins discussing the cetacean stomach but the entire paragraph centers more around cetacean sensory structures. I would suggest either removing the sentence on the digestive system or giving it its own section, if relevant, and devote a paragraph to the sensory system aspects.

On the section on heterochronic processes in cetacean development, and throughout the thesis, greater precision is needed in evolutionary terminology. For example, one sentence states '*C. marginata* is not a paedomorphic species', but species themselves cannot exhibit paedomorphism – only individual traits can be paedomorphic. In this example, rewording could be to: '*C. marginata* does not possess paedomorphic traits'. Despite these minor organizational and terminological issues, Chapter 1 provides a solid foundation for the work that follows.

Chapter 2: Chapter 2 outlines the materials and methods for each of the thesis chapters. The breadth of the methods used for the thesis is exceptional, incorporating morphological, phylogenetic, molecular, and developmental techniques in a holistic approach. In terms of the study on suture ossification, Ms. Telizhenko described examining several proxies: degree of ossification of sutures, number of fully ossified sutures, and presence of accessory bony elements and clefts. The use of different phylogenetic signal metrics appropriate for different data types (D for binary characters, Blomberg's K and Pagel's λ for continuous traits) demonstrates methodological rigor, although it might be worth explicitly mentioning the latter in the methods. The abbreviation 'ASR' appears for the first time in the 'Phylogenetic tree reconstruction' section without being defined – this should be spelled out on first use (presumably 'Ancestral State Reconstruction'). While the molecular and genomic/genetic methods are beyond my

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primary expertise, the morphological and phylogenetic approaches appear sound and appropriate for the research questions. Overall, the methodological framework appears comprehensive and well-suited to address the research questions, though some minor clarifications would improve accessibility.

Chapter 3: This chapter presents the results of a published study on cetacean skull evolution/evolutionary rates and ossification patterns. While the research is substantial and well-executed, the presentation could be improved with better organization and clearer terminology. The chapter begins with methodological details rather than providing a brief motivation statement about why this research is important, which would help orient readers to the significance of the work before diving into results. Additionally, early paragraphs discussing skull maturity could be clearer about whether they describe the literal developmental/ontogenetic stage of specimens examined or the results of the ossification analysis performed.

Several terminology and clarity issues affect readability throughout the chapter. The phrase "least ossified sutures" is particularly problematic, as it's unclear whether this refers to fewer total sutures being ossified or to the degree of ossification. Using "degree of ossification" consistently would improve clarity. Similarly, the discussion would benefit from reordering suture-related topics or explicitly defining terminology to help readers follow the analysis more easily. The text also contains imprecise evolutionary terminology, such as describing "the suction feeding mammalian phenotype is obviously paedomorphic" (should be "complex of characters" rather than "phenotype") and referring to "particularly paedomorphic morphotypes" (better stated as species "comprising a large number of paedomorphic traits").

Despite these presentation issues, the chapter contains excellent scientific content. The discussion of accessory bones, fontanelles, and clefts as evidence for delayed ossification is particularly well done and clearly reasoned. The discussion effectively highlights the study's importance for understanding cetacean evolution. Technical improvements needed include correcting the spelling error from "*Olimpicetus*" to "*Olympicetus* spp." in Table 2 and enhancing Figure 11 with better explanation of the color scheme and highlighting of species discussed in the text. Overall, this represents solid research that would benefit from clearer presentation and more precise terminology.

Chapter 4: This chapter describes results of another published paper on the evolution of genes regulating limb development and their relationship with forelimb variation in cetaceans. Like the previous chapter, it begins with a short description of methodological details and would benefit from a brief introduction describing the motivation. The work is very interesting, and I only noticed a few technical issues. For example, the singular of 'phalanges' should be 'phalanx'. Further, the sentence 'Therefore, increase in phalangeal count in certain digits is paralleled by decrease in other digits' is slightly unclear – is it a decrease in the digits themselves, or other phalanges meant here? Based on the prior sentence it seems like 'digits' might be the intended meaning, but it would be good to clarify. The summary describing the trends in the evolution of limb-related genes is especially good, and it made me think a summary figure would be very interesting to include and complement it. There is already a very nice summary figure complementing the following paragraph, but I think it would really help a reader follow the discussion if there were another summary figure for the prior section. The wording of this sentence "Although there is clear evidence of *EVX1* expression in the limb bud, its function in the development of limb remains is not fully understood (Niswander & Martin, 1993)." is unclear, consider revising. The summaries at the end of the chapter are very good and clearly highlight the importance of the work, identifying a diverse set of regulatory genes that code transcription factors involved in pathways leading to distinct phenotypes.

Chapter 5: This chapter describes results from another published paper, this time on the selection processes related to the genes regulating limb development as they relate to the humerus specifically. The chapter begins similarly to the others, although it is clearer that it is a statement of what was done before diving into the results. It would still benefit from a brief statement introducing the chapter and its importance and relevance to the rest of the work. This chapter is very well done, and in particular Figure 33 is visually appealing and effective. I only have a one technical comment: on page 118, '...transited...'

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should be 'transitioned'. The discussion and final paragraph effectively highlight the importance of this work, including the continued theme of relaxed selection in cetacean evolution – in this case specifically for humerus development – with appropriate caveats noted for future analyses.

Chapter 6: This is the chapter containing unpublished work on the analysis of expression of limb-related regulatory genes in odontocetes. The introductory paragraph is good; it states the methods while also briefly mentioning novel results. It could still use a brief description of the motivation or brief introduction, but it is overall clearer what is happening before diving into the rest of the results. The work is very interesting, and again I only have a few technical notes. For example, the common names 'harbor porpoise and northern bottlenose whale' are used once and it is not indicated which species these are as species names are used throughout. It would therefore be good, if common names are used, to also indicate their species names at first mention. The result that there is high expression of *HOTTIP* in most tissues is very intriguing, and the relevance to the lack of cancer observed in cetaceans is also very interesting. Ms. Telizhenko mentions the small sample size as a downside of the work, but the results represent a good foundational phylogenetic bracket with species from crucial and informative parts of the tree (delphinid, phocoenid, and ziphiid). The ziphiid results are particularly interesting from this perspective, and the existing sample can also help guide a targeted strategy for future sampling (obviously still opportunistically).

Conclusions: The conclusions summarize the findings of each chapter very well, but I would like to have seen a synthesis of the common and connecting themes of the whole work. Conclusion number 5 definitely hints at it and is a very good insight, effectively identifying the overarching evolutionary pattern: "The relaxed selection in gene evolution combined with insertions and deletions in regulatory genes and positive selection on specific sites, seems to be an important evolutionary pattern driving aquatic adaptations of mammalian limbs." This represents exactly the kind of synthetic thinking that could have been developed further to integrate the findings across all chapters.

The individual conclusions clearly demonstrate the breadth and quality of the research, from delayed skull ossification patterns to novel gene expression patterns in adults. The thesis would be strengthened by explicit integration of how the different lines of evidence examined – morphological, phylogenetic, and molecular – converge on a unified understanding of cetacean developmental evolution. The work clearly reveals a coherent story about how relaxed selective constraints on developmental genes enable the morphological innovations characteristic of cetacean evolution, including both the delayed cranial ossification and limb modifications documented in the different chapters. Making these connections explicit in a synthetic conclusion would highlight the significant contribution this work makes to understanding the developmental mechanisms underlying major evolutionary transitions.

Conclusion: In conclusion, I find that the PhD thesis by Valeriia Telizhenko represents a substantial contribution to evolutionary biology, executed at the appropriate scholarly level and ready for consideration by all relevant academic bodies. I am convinced it merits the doctoral degree, and I warmly recommend its acceptance as a worthy doctoral contribution.

Yours sincerely,

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