Review

of the Dissertation by Telizhenko Valeriia "Genetic and Morphological Proxies for Evolutionary Changes in the Ontogeny of the Cetacean Skeleton", submitted for the degree of Doctor of Philosophy in the specialty 091 – Biology

Cetaceans are among the most iconic groups of vertebrates, whose evolution involved dramatic changes in both habitat and body structure. These transformations have long attracted scientific interest, particularly regarding modifications to the skeleton linked to a fully aquatic lifestyle. While skeletal changes in extinct and extant cetaceans have been extensively studied, the genetic–morphological interplay remains poorly understood. Valeriia's dissertation makes a significant contribution to addressing this knowledge gap and is of high relevance.

The dissertation consists of 220 pages. Its structure is traditional and includes an abstract, a list of the author's publications, an introduction, a literature review, a section on materials and methods, four chapters presenting the author's results and discussion, conclusions, and 10 annexes. The materials are illustrated with 38 figures and 7 tables. The bibliography contains 397 references.

The literature review is informative and presents a comprehensive overview of cetacean characteristics, including their distinctive morphology and physiology shaped by aquatic adaptation. Significant attention is paid to genetic regulation in cetacean ontogeny. The author clearly defines unresolved issues, particularly the incomplete understanding of cranial adaptations and the genetic mechanisms underlying skeletal transformations.

The "Materials and Methods" section specifies the number and origin of skulls and forelimbs studied. Notably, the author used specimens from European and American museum collections, broadening the taxonomic scope. Comparative analysis with related mammalian groups, especially artiodactyls, allowed for a more comprehensive evolutionary assessment. The inclusion of ontogenetic material, though limited, enables the analysis of skull ossification dynamics.

This section also outlines the limb-regulated genes analyzed and the statistical methods used to test associations between gene sequences and forelimb morphology. It is particularly noteworthy that the author used not only genomic and protein sequences from available databases, but also personally conducted laboratory studies of gene expression in flippers, dorsal fins, and flukes. A wide array of statistical tools was applied, including phylogenetic analyses, ancestral state reconstructions, models of evolutionary rates, and selection pressure estimations, contributing to the robustness of the conclusions.

Chapters 3 to 6 present results and discuss them. The content is logically structured and clearly written. Chapter 3 reports a quantitative assessment of cranial suture ossification and accessory bone formation in modern whales. The author identified a low rate of postnatal suture ossification, which was correlated with the presence of accessory elements and cranial clefts, and observed a strong phylogenetic signal in cetacean cranial development. Importantly, accessory cranial bones are interpreted as possible evolutionary reversals or developmental novelties, diverging from the trend of cranial simplification in mammals (Williston's law).

Chapter 4 explores forelimb morphological diversity, investigates selection across 25 limb-regulated genes, and tests gene-morphology correlations. This integrative approach is innovative and holds potential for broader application in vertebrate studies. The author detected relaxed selection in key regulatory genes involved in phalange and wrist bone development, as well as non-synonymous substitutions in BMP2 and alanine repeat variation in HOXD13 in taxa with unusual limb morphology and aquatic lifestyles.

In Chapter 5, selection analysis of 11 genes implicated in humerus development revealed relaxed purifying selection in several regulatory genes, including PTCH1, GLI3, and PBX3. Additionally, two positively selected sites were identified in GLI3 in *Kogia breviceps*. The conclusion that a combination of relaxed and positive selection shaped humeral diversification in cetaceans is well-supported.

Chapter 6 describes gene expression analysis of posterior Hox genes in juvenile, adult, and senescent odontocetes. The author reports persistent postnatal expression in flippers, dorsal fins, and tail flukes—a novel finding not observed in other mammals.

The dissertation includes high-quality visual material that effectively illustrates skull and limb morphology, phylogenetic trees, and ancestral state models.

The amount of material analyzed, the application of modern research methods, and the originality of the findings make the dissertation reliable and the conclusions justified.

I suggest the author address the following questions in more detail:

1. The author focuses on postnatal development in the dissertation. However, many morphological traits are determined embryonically. Can the author's findings be extrapolated to embryonic development?

2. Given that certain genes remain active into adulthood in cetaceans, unlike in other mammals, how might this extended expression influence limb morphology?

3. The term "re-emergence" is used regarding ossification centers in cranial development. Could the author clarify this usage, considering that evolution typically does not restore lost structures, but maybe replaces them with analogues or transformed derivatives?

The dissertation by Valeriia Telizhenko represents a completed, independent study on a relevant topic. The scope and depth of the research meet the requirements for the Doctor of Philosophy degree. The findings are of theoretical and practical value and exhibit a significant degree of novelty. The results have been presented at major conferences and published in peer-reviewed journals, including three articles indexed in the Web of Science Core Collection and Scopus (Q1).

Based on the above, I recommend awarding Valeriia Telizhenko the degree of Doctor of Philosophy in the specialty 091 – Biology.

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