

REVIEW

of the manuscript "**Genetic and morphological proxies for evolutionary changes in the ontogeny of cetacean skeleton**"

submitted as the dissertation for a scientific degree of the Doctor of Philosophy on a speciality 091 – «Biology» by **Valeriia Telizhenko**

The manuscript presents the results of a study of separate peculiarities in the evolution of Cetacea. The study combined conventional methods of morphological investigations with rather up-to-date methods of phylogenetic analysis, statistical analyses, and bioinformatics. The investigation was based on large samples of studied materials (434 skull and 145 limb specimens) and extensive datasets.

The results obtained in the study are original, significant, and have a high degree of scientific novelty; they add new and important information to the knowledge about cetacean evolution, as well as some new data about the evolution of mammals in general.

The manuscript is written in clear and elegant scientific English; I have no comments on the style and grammar of the text. It includes an Annotation, a list of papers published by the author, an Introduction with all necessary parts and sections (aim, objectives, brief description of methods, acknowledgements, etc.), six chapters, Conclusions, References, and Annexes.

The first chapter, LITERATURE REVIEW, at the beginning presents brief but comprehensive information on cetacean anatomy, biology, and physiology. A separate section (1.3) deals with published information about the heterochrony in cetacean development; the next two sections review the information about genetic regulation of ontogeny, cetacean evolutionary genetics and development. Thus, the latter three sections are directly related to the subject and objectives of the study.

Comments:

1. There are no authorities' names and years of descriptions in animal scientific names when they are first mentioned in the text.

2. I would suggest finishing each section (sub-chapter) with a summary or conclusion highlighting the gaps in published information on each subject. Such a summary is present only at the end of the last section.

Chapter 2, MATERIALS AND METHODS, gives a concise but comprehensive description of the studied materials and various methods used in the study, morphological and phylogenetic analyses, molecular studies, quantitative analyses, along with the names of corresponding programs and R packages.

Chapters 3–6 present the results of the study; each contains a Discussion section after the description of results.

Chapters 3. EVOLUTION OF ACCESSORY BONES AND OSSIFICATION OF THE CETACEAN SKULL. This analysis is based on a large and representative sample comprising 47 cetacean and 15 other mammalian species. Peculiarities of the skull anatomy in cetaceans (suture ossification, accessory bones, fontanelles and clefts) and related groups of mammals are considered from a phylogenetic viewpoint. The descriptions are supplemented with photographs of good quality.

Comments:

1. "Wormian bones" (first letter capitalised) or "wormian bones"? Both variants of spelling are present in the text.

2. There are many abbreviations in the text of this and other chapters. Their meaning is explained only in "Materials and Methods"; sometimes it is difficult to find this information. It would be convenient, in my opinion, to give a separate list of abbreviations at the beginning of the manuscript.

Chapter 4. EVOLUTION OF GENES REGULATING LIMB DEVELOPMENT GIVES CLUE TO THE VARIATION IN DISTAL FORELIMB MORPHOLOGY OF CETACEANS

The chapter presents the results of molecular phylogenetic analysis based on concatenated alignment of 100 coding sequences (4.1) and its correspondence to the details of forelimb anatomy, including the ancestral state reconstructions for

particular parts of cetacean forelimbs (4.2). The selection patterns in the evolution of limb-regulating genes are analysed; the results are especially interesting and important, in my opinion, since the observations and conclusions are expanded on various aquatic mammals, other than cetaceans, identifying "several trends in the evolution of limb-related genes".

Comments:

1. I noticed incorrect formatting of several references in the text. Perhaps that was the fault of the reference manager used by the author.

Chapter 5. SELECTION PROCESSES IN THE GENES REGULATING HUMERUS DEVELOPMENT. The smallest chapter (4 pages of results and 4 pages of discussion) deals with the search for positive selection in the evolution of regulatory genes.

Comments:

1. The only inaccuracy I noticed is an incorrect reference to Fig. 33 (referred to as "Fig. x").

Chapter 6. ANALYSIS OF EXPRESSION OF LIMB-RELATED REGULATORY GENES IN ODONTOCETES. The author "identified and quantified the expression of posterior HoxD genes, HOXA13 and HOTTIP, in the connective tissues of the flippers, dorsal fin, and fluke of *H. ampullatus*, *P. phocoena*, and *T. truncatus*. Additionally, we successfully measured their expression in the fluke skin of *H. ampullatus* and *P. phocoena*, as well as in the carpal cartilage of *H. ampullatus*." The most interesting and important, in my opinion, part of the chapter is a separate section in the discussion "Hox-genes in oncogenic processes". The possible role of HOXD10 (positive) and HOXD11 and HOXD12-13 (negative) is discussed. The author concluded that "The expression of HOXD10 detected in the connective tissues of the harbor porpoise and northern bottlenose whale in our study could indicate an overall higher expression of this gene in whales and might provide clues to cancer resistance in cetaceans."

Conclusions in the manuscript correspond to the objectives of the study and summarise the main results. The fifth conclusion is extremely interesting, since it

is important for our understanding of particular details in the limb evolution of aquatic mammals.

Comments:

1. Conclusions #3 and #6 are excessively extended, in my opinion. Some details presented there seem to be unnecessary in the conclusions.

Conclusion. The dissertation is the author's original scientific investigation performed on sufficient material, with important and interesting results and a high degree of scientific novelty. The results were included in three scientific papers published in international journals of high rating and presented at several international scientific conferences. In my opinion, Valeriia Telizhenko possesses a high level of qualification as a researcher, and, with no doubts, can receive the scientific degree of the Doctor of Philosophy in the speciality 091 – «Biology».

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