External Assessment (Review)

of doctoral thesis submitted by

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entitled

Molecular characterisation of parvalbumin gene: evolutionary insights and forensic applications for fish species identification and authentication

I accepted with interest for consideration a dissertation that deals with a complex study of parvalbumin gene, its evolution and role in studies of alergens, as well as in species identification.

I hereby declare that I do not have any relationship to the candidate that would preclude me from accepting a review of this dissertation, and that there is no conflict of interest.

The doctoral dissertation, submitted as a set of scientific papers with introductory and conclusive commentary, consists of four articles published in international scientific journals with IF (the candidate was the first author in all of them) and is topically complemented by one first-authored manuscript under review. The papers published have already undergone the review procedure in journals Genes (IF = 3.5), Fishes (IF = 2.3), Journal of Food Composition and Analysis (IF = 4.3) and European Food Research and Technology (IF = 3.3) and being a thesis reviewer, it was not my task to reassess them.

The introductory part describes parvalbumins (*pvalb*) as calcium-binding proteins highly abundant in fish muscle depending on species or, rather on proportion of red or white muscle tissues. The evolution of *pvalb* gene in teleosts is reviewed with respect to whole genome duplication events and next subsequent evolutionary processes of the duplicated genes. Last, the importance and applicability of species identification methods is pointed out in light of increasing aquaculture production and utilization in trade legal cases.

The aim of the dissertation was to characterise the *pvalb* gene, describe the evolution of *pvalb* genes in teleosts upon genomic and transcriptomic evidence, identify their types and genetic changes, with potential implications for the adaptive evolution of teleosts. The goal was also to investigate the potential of the *pvalb* gene analysis as forensic tool in fish species detection upon qPCR quantification. It is evident from the particular chapters, that the objectives were fulfilled and the work has yielded a number of interesting findings applicable in the next genomic studies, to understand functional aspects of *pvalb* like its allergenicity, as well as in fish food industry. Very interesting is the description of a novel genomic primer and proposal of a qPCR assay to detect and distinguish both species of the genus *Lophius*. When published, the developed universal but fish-specific primer targeting *pvalb* β gene will surely deserve a great attention and use.

I have some small partial comments on this work which in no way undermine the scientific contribution of the whole thesis.

In Chapter 1 the author wrote on pg. 20: „…several teleost lineages such as the Salmoniformes, Cyprinidae, and Sturgeons underwent additional lineage-specific duplication events…" but sturgeons should not be considered a teleost lineage. They are bony fishes (Osteichthyes), belong to class ray-finned fishes (Actinopterygii) but to superorder Chondrostei, not Teleostei. On the other hand in Chapter 2, pg. 52 sterlet (a species of sturgeon) is already correctly described as „a non-teleost fish species". When associating the rise of more clusters of *pvalb* gene with genome duplication events, one might expect higher diversity of *pvalb* genes in sturgeon species that have undergone more than one such additional WGD event as sterlet did, i.e. two or three like *A. baerii, A. gueldenstaedtii, A. transmontanus* or *A. brevirostrum*. I am just curious, is there anything known on their *pvalb* genes diversity?

I have the following question for the candidate and due to the announced overlap of the dissertation defence date with date of my university´s scientific board meeting, I ask the committee members to kindly consider the candidate's answer in my absence:

With the principle having been known already earlier, the DNA barcoding method was established some 11 years ago for species identification and became widely used for fish species identification, ecological monitoring, invasive species monitoring, etc., as well as in food safety, including forensic applications. Along with later modifications (eDNA, metabarcoding), it is now used for species molecular identification using large reference libraries. What are the advantages and disadvantages of the candidate's proposed method compared to this approach?

I am convinced that the dissertation thesis of the candidate Subham Mukherjee definitely meets the requirements for a scientific qualification thesis of this level. Therefore, I evaluate the thesis positively, I agree that the thesis should be accepted for defence and after its successful defence the academic title "Ph.D." should be awarded to the nominee.

Vodňany, February 5, 2024

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