



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Mujahid Ali Shah, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: Sturgeon (Acipenser) represents the evolutionary transition from holoblastic to meroblastic cleavage pattern and unique gut development	

REVIEWER:

Surname: Krylov	Institution: - Univerzita Karlova, Praha
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Please describe your professional relationship to the PhD student: NONE	Please describe your field of expertise: Developmental biology

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

Sturgeon altogether with Bichirs represent interesting model organisms for the evolutionary studies of switch from holoblastic to meroblastic embryonic cleavage. Yolk utilization by the embryo and larvae and the contribution of vegetal yolk rich blastomeres to the formation of digestive tube are the consequent fundamental questions of developmental biology important for the understanding of the emergence of extraembryonic tissues in Amniotes. From this point of view the PhD thesis and two included papers and one manuscript represent an important contribution to this field with high impact for the basic research.



Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (**extent ¼ – ½ page**):

PhD thesis is written on thirty-five pages including references. The structure and ordering are correct. The first chapter represents a general introduction where author described basic developmental processes during early embryogenesis of holoblastic (*Xenopus*) and meroblastic (*Zebrafish*) model organisms. Next chapter is focused on the sturgeon development regarding the evolutionary switch between holoblastic to meroblastic cleavage. RNA localization and distribution important for the differentiation of mesodermal tissues and determination of primordial germ cells is compared among holoblastic and meroblastic species with emphasis on amount and distribution of yolk. Consequently, author focuses on the development of endodermal structures such as gut. The last chapter is aimed on techniques used for the inhibition of blastomere cleavage important for the determination of the role of vegetal blastomeres in the nutrition and contribution to gut development in sturgeon. Author then described three objectives: 1) Development of effective technique for the inhibition of specific blastomere cleavage, 2) using this technique to perform the functional study to determine the fate of vegetal blastomeres in sturgeon, i.e. if they contribute to the development of gut (as in *Xenopus*) or serve only as a nutritional deposit (as in *zebrafish*), 3) comparison of gut development among holoblastic and meroblastic taxa and sturgeon laying between these two groups. All three aims fully correspond to the content and structure of PhD thesis and to included two papers and one manuscript.



OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

The PhD thesis is focused on the sturgeon (Acipenser) as a unique model organism for the study of the evolution of holoblastic and meroblastic embryonic cleavage and the formation of digestive tube. Thesis is based on two impacted publications and one manuscript. In all cases the author is on the first position. As mentioned above, the thesis is written on 35 pages including references. The structure and chapter ordering are logic and fully correspond to the set goals and included papers and manuscript. The text is well written, understandable and with minimum typos or grammatical errors except included manuscript which needs some language proofreading. The first paper "Novel technique for definite blastomere inhibition and distribution of maternal RNA in sterlet *Acipenser ruthenus* embryo" published in the journal Fisheries Science (IF = 1,617) describes a novel approach for the inhibition of specific blastomere cleavage for the subsequent study of the contribution of vegetal blastomere to the gut development and/or only for the nutrition. The method utilizes microinjection of diatom derived toxin 2,4 – decandienal (DD) into vegetal blastomere and subsequent focused visible light irradiation (44.86–91.15 W m⁻² + 395-700nm). In the second paper "Blastomeres derived from the vegetal pole provide extra-embryonic nutrition to sturgeon (Acipenser) embryos: Transition from holoblastic to meroblastic cleavage" (Aquaculture, IF = 4,67) author confirms the intermediate position of sturgeons between holoblastic amphibians (*Xenopus*) and meroblastic teleost (zebrafish). Cleavage inhibition of vegetal blastomeres by DD and light irradiation resulted in the normal gut development indicating the only nutritional role of vegetal blastomeres similarly as yolk syncytial layer in zebrafish but in the cellular form as in *Xenopus*. As expected, treated embryos were sterile since primordial germ cells are derived from vegetal blastomeres. The third PhD thesis output in the form of the manuscript: "The gut development of sturgeon is unique among vertebrates: A comparative study" revealed the unique gut formation as an endodermal tube continuously surrounding the yolk cell mass for the subsequent digestion and utilization. In *X. laevis* it was shown that the endodermal yolk cells are important not only for the nutrition but also for the gut formation. The manuscript text is relatively full of grammar errors which it is in the contrast with entire PhD thesis and published articles where I noticed their minimum. The chapter general discussion clearly summarizes obtained results and discusses them in the wider context of developmental biology studies concerning early embryonic development of selected Anamnia and Amniota species. In conclusion the PhD thesis represents a great contribution to the puzzle of basic research of body plan patterning and formation with respect to embryonic but also extraembryonic tissue determination and differentiation. I fully recommend the PhD thesis for the defence, and I have three questions:

- 1) What do you think it was the first during the transition from holoblastic to meroblastic cleavage? The growing amount of yolk resulting in the formation of YSL as in zebrafish or formation of YSL allowing the accumulation of higher amount of yolk for the embryo nutrition?
- 2) It seems that yolk acellularization or just starting this evolutionary process and being between holoblastic and meroblastic species as sturgeon is accompanied with the mesoderm



determination from the animal pole and not from vegetal pole as in *Xenopus*. Would it be possible that this shift is caused by the microtubule “network failures” during the formation of syncytial yolk and thus mesoderm fate signalization had to be “transported” from acellular vegetal to cellular animal pole? If so, couldn’t be the holoblastic cleavage of ventral part of sturgeon embryo secondary derived from the acellular state? So that sturgeons were meroblastic with the mesoderm determination from the animal pole and secondary developed a holoblastic cleavage of ventral blastomeres?

- 3) The emergence of yolk sac was perfectly described in the PhD thesis because of the accumulation of enormous amount of yolk. However, what about the allantois, amnion and chorion? Are these extraembryonic tissues tightly coupled with the emergence yolk sac or at least amnion and chorion developed lately (intermediate state between Anamnia and Amniota)?

FINAL RECOMMENDATION

- ☒ PhD thesis can be recommended for defence
☐ PhD thesis can be recommended for defence with reservations
☐ PhD thesis cannot be recommended for defence



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7.7.2022, Prague

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Date and place

Vladimír Krylov

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Name and signature