



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Kseniia Pocherniaieva, M.Sc.	First name(s), surname, titles of supervisor: Dipl.-Ing. Vojtěch Kašpar, Ph.D.
Title of PhD thesis: The foundation of maternal factors in sturgeon: from oocyte to embryo	
REVIEWER:	
Surname: Kowalski	Institution: Polish Academy of Sciences in Olsztyn, Poland
Name: Radoslaw	
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Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: Fish reproduction, spermatology

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**):

The sturgeons are animals important for both, science and practice. Scientifically they are living fossils and practically, source of one of the most expensive foods: caviar. Therefore, studies involving these animals are of high importance in the world fisheries science field. Recent advances in PCR techniques allows us to describe life in a very detailed manner. PhD candidate in her work use PCR tomography after applications of the panel consist of 12 reporter genes primers. The genes were: beta-actin (actb), peptidylprolyl isomerase A (ppia), 5'-aminolevulinatase synthase (alas1), succinate dehydrogenase complex flavoprotein subunit A (sdha), tyrosine 3-monooxygenase (ywphae), DND microRNA-mediated repression inhibitor 1 (dnd), DEAD-box helicase 4 (vasa), vegt protein (vegt), wingless-type MMTV integration site family member 11 (wnt11), DEAD-box helicase 25 (ddx25), orthodenticle homeobox 1 (otx1), and growth differentiation factor 1 (gdf1). The very well structured organisations of panel (good choice of the reporter genes) and applications of eggs sectioning indicates, that PhD candidate has a solid scientific background and is ready to answer many more questions in biology science. She also uses fluorescence micrographs which apart is relatively simple, but also effective methods to visualisations specific structures in living organisms. Presented work is well connected with the latest achievement of the international team that originated from University of South Bohemia. In recent years, this team become a world leader in sturgeon science. In this context, presented PhD thesis is well incorporated into recent achievements of the fisheries science.



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**):

Structure of the thesis is clear. The chapters are in good orders providing a clear „road map“ of the thesis. The introduction part is divided into sections which well describes the background of the study and explain its importance. Two publications consist „the core“ of the thesis are published in journals with international recognitions. The general discussion provides a comprehensive synthesis of the main results obtained in presented manuscripts. The conclusions are clear and indicate the scientific maturity of the candidate. Apart from that, I identified some minor editing problems:

1. Page 16 Line 7 – crucial instead of crucial.
2. Page 17 Line – Double use of „in“
3. Page 56 Line 27 – approche should be approach
4. Page 56 Line 29 – embryos should be embryo
5. Page 58 Line 35 – „the he“ should be the

In their work, PhD candidate aimed to exhibit distribution maternal factors of sturgeon egg responsible for the formation and migration of PGCs, together with the identification of transition points. As a result, the authors expect to better understand gametogenesis, sex differentiation, PGCs evolution and basics of normal development in sturgeon. To investigate these problems following the aims of the study was pointed:

1. To reveal the fundamental architecture of maternal messenger RNAs in sturgeon oocyte via the method of quantitative polymerase chain reaction tomography.
2. To clarify the origin of germplasm in the body plan of sturgeon oocyte and evaluate the impact of germplasm localization on germ cells fate.
3. To characterize the transition from maternal control to activation of the zygotic genome in sturgeon embryos.
4. To identify the point of mid-blastula transition in sterlet *Acipenser ruthenus* and *A. ruthenus* x *Acipenser gueldenstaedtii* hybrid.
5. To compare mRNA profiles in sturgeon with available patterns in the frog oocyte.

The obtained results, well correspond with presented goals and allow the PhD candidate to draw 7 conclusions among which we can find the answers to pointed in the introduction questions.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Embryo development is the process in which all living organism shows highest similarities to each other. Such a life window is the best object to demonstrate the evolutionary mechanism and directions, thus helping to understand the origin of life. Studies regarding early life development in sturgeons are important due to both, scientific and practical reasons.

Scientifically it is important due to Acipenseridae classification into the living fossils, thus helping us to get insight into life history on the Earth. Its comparison to other animals, like anurans or reptiles, shows high usefulness in understudying the processes driving the evolution.

The practical importance of such a study is related to the higher-priced product such as caviar which sturgeons are the source of. In this field, the highest-priced caviar originated from the Beluga, is quite difficult to harvest due to the long maturation process of beluga female and difficulties in hatchery manipulations with fish weight over 100kg. The recent advance in primordial germ cells (PGC) transplantations makes the dream of “living bioreactors” producing caviar, possible to become true. Use of starlet as a vehicle of beluga PGC is already well-investigated problem by the scientific team of PhD candidate. In such a context, the presented research shows complementarity within the broader research conducted at the University of South Bohemia.

Taking together, presented PhD thesis, shows high value in the area of fisheries since as well as the evolutionary field. The PhD candidate aimed to exhibit distribution maternal factors of sturgeon egg responsible for the formation and migration of PGCs, together with the identification of transition points. As a result, the authors expect to better



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The first manuscript describing answers to presented questions was published in *Journal of Experimental Zoology part B Molecular and Developmental Evolution*. In this publication, PhD candidate uses quantitative polymerase chain reaction tomography method after applications of 12 reporter genes primers. The genes were: beta-actin (*actb*), peptidylprolyl isomerase A (*ppia*), 5'-aminolevulinic synthase (*alas1*), succinate dehydrogenase complex flavoprotein subunit A (*sdha*), tyrosine 3-monooxygenase (*ywhae*), DND microRNA-mediated repression inhibitor 1 (*dnd*), DEAD-box helicase 4 (*vasa*), vegt protein (*vegt*), wingless-type MMTV integration site family member 11 (*wnt11*), DEAD-box helicase 25 (*ddx25*), orthodenticle homeobox 1 (*otx1*), and growth differentiation factor 1 (*gdf1*).

Thanks to results authors were able to drive important conclusions among which I found 3 worth to highlight.

The authors conclude that the changes in morphological geometry during development might be due to the higher yolk volume in the sturgeon oocyte. Thus, authors hypothesize that *vegt*, which induces mesodermal differentiation in ectodermal regions in *Xenopus*, performs the same function in sturgeon, but with a slight shift to the animal region. Since sturgeons are more ancient animals than *xenopus* I would rather say, that *vegt* get slight shift to the vegetal region in *xenopus*. In my opinion, this observation shows some importance to reveal evolution mechanisms.

Another important finding is that in contrast to *Xenopus*, sturgeon embryo blastomeres cannot be separated into a dorsal and a ventral group, with an equal number of animal and vegetal cells in each. In my opinion, such a primitive (unorganised) localisation corresponds with an ancient origin of sturgeon family and indicated, that evolution drives the living organisms from the chaos into higher levels of organisations at the earlier ontogenesis steps.

The authors also show that localization of germplasm determinants in sturgeon was similar to profiles in *Xenopus*, thus demonstrating the similarity of germ cell specification in sturgeon to that of anurans. Thus, they conclude that localization of maternally derived germplasm determinants is characteristic of the preformation mode in both sturgeon and anurans. I would like to add that it also shows that the "foundation of life" is conservative among animals embryogenesis which might indicate, that its organisations were a primary and most important task in the evolution processes. Most probably, such a stable localisation of the "life foundation" is prerequisite for further evolutionary development.

The second manuscript aimed to identify the point of mid-blastula transition in sterlet *Acipenser ruthenus* and *A. ruthenus* x *Acipenser gueldenstaedtii* hybrid Mid-blastula transition. To achieve this task, PhD candidate uses hybrid as a model to compare its development to the maternal species origin. The only weakness of this part is lack of the investigation the paternal species (Siberian sturgeon) to compare its embryogenesis to a hybrid one. Since differences were found between sterlet and hybrid embryogenesis it is likely, that some "male origin" consequences in embryogenesis could be identified and increase the scientific soundness of the manuscript. Apart from this criticism, manuscript in the present form is a valuable part of sturgeon embryogenesis knowledge and did diminish the overall, good assessment of the PhD candidate work.

Judging the candidate activities on the field of science it is also worth to mention, that Kseniia Pocherniaieva participated in numerous scientific projects and published 6 manuscripts listed by the Scopus database. According to the Scopus database, his Hirsh index is equal 2 which is very good results as for young researcher on the early (the first) step of development.

Despite all of my criticisms towards presented Ph.D. thesis, its overall scientific value is high. It is my pleasure to recommend Kseniia Pocherniaieva as a successful Ph.D. candidate.



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Czech Republic

FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
- PhD Thesis can be recommended with reservations for defence
- PhD Thesis can not be recommended for defence

10.07.2020 OLŠETYN

Date and place


Radosław Kowalski

Name and signature