



Confidential

Supervisor's Review of USB RIFCH PhD Thesis

Surname of the PhD student: Lebeda, MSc.	Name of supervisor: prof. Ing. Martin Flajšhans, Dr.rer agr.
Title of PhD thesis: Optimization of chromosomal manipulations in acipenserids	

OVERALL COMMENTARY ON THE PhD THESIS

Ievgen Lebeda entered my lab in 2010 with MSc. in biophysics and his task was to develop methods of chromosomal manipulations in sturgeons, especially methods of gynogenesis. Ievgen, with his silent discreet independence became familiar with recent techniques of DNA inactivation in gametes, artificial reproduction, egg incubation and progeny nursing in sturgeons, methods of somatic ploidy restoration in gynogenesis, as well as with assays to analyse the damaged DNA, to study DNA photoreactivation process and with flow cytometry for testing the success of gynogenesis. Moreover, he extended his initiative to alternative chemical methods of DNA inactivation in gametes and recently, also to attempts to produce fish with diploid gametes. He spent the mandatory 3 months of stay abroad at the University of Warmia and Mazury in Olsztyn, Poland (Prof. D. Fopp-Bayat) where he improved his skills in chromosomal manipulations and molecular genetics of sturgeons. During his studies, he also completed four courses, mainly in scientific communication, statistics and image analysis. He participated in four international symposia in Europe and in one in Canada, presenting four lectures and two posters.

Ievgen Lebeda proved his pedagogical abilities mostly in field- and lab training of MSc. students, lab demonstrations for student excursions and he often taught other PhD students to use fluorescence imaging, Comet assay and flow cytometry.

His PhD thesis is entitled The optimization of chromosomal manipulations. Despite the fact that he dealt mostly with different aspects of gynogenesis, he truly projected in the thesis all the due chromosomal manipulation techniques, as gamete DNA inactivation, somatic ploidy restoration, polyploidy induction, etc. The thesis itself is conducted as a compilation of two papers published in peer-reviewed IF – journals (Chapters 2 and 3), with the introductory review on the problems of chromosomal manipulations and particularly of sturgeon gynogenesis (Chapter 1), further



accompanied with submitted manuscripts under review on DNA photoreactivation after sperm irradiation for gynogenesis in sterlet (Chapter 4) and on the use of flow cytometry for assessment of success of gynogenesis using heterologous sperm of sturgeon species differing with ploidy level (Chapter 5). The last Chapter 6 deals with very recent first (and thus yet unpublished) results on optimization of tetraploidy induction, with the idea to obtain potential fish with diploid gametes, to overcome mortality issues associated with somatic ploidy level restoration. The following general discussion attempts to summarize the progress gained in sturgeon gynogenesis, especially the effects of UV irradiation for DNA inactivation and of its alternatives, and highlights some points of future research of sturgeon gynogenesis.

I can see the most important milestones of progress achieved in work and thesis of levgen Lebeda as follows:

- 1) He published finding an optimally low UV dosage for DNA inactivation in sturgeon spermatozoa, described a reliable protocol of this technique but also noted the problems associated with high UV absorption in sperm. He also described conditions of irradiated sperm preservation and handling, under which the DNA photoreactivation is avoided.
- 2) He developed a method of large-scale induction of gynogenesis in sturgeons with survival rate sufficient for aquaculture.
- 3) He described and verified the hypothesis of flow cytometric sorting the putative gynogenetic progeny after fertilization with DNA-inactivated sperm of species differing with ploidy level, the so-called „interspecific gynogenesis“.

As a supervisor of levgen Lebeda, MSc., I can state that the aims of this PhD thesis were fulfilled and that levgen, in his silent and inconspicuous way came out with several novel findings which enriched the field of sturgeon chromosomal manipulations.

Finally, I recommend his PhD thesis for defense.



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FINAL RECOMMENDATION

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

Vodňany, May 6, 2014.....
Date and place

Martin Flajšhans
surname and signature