



**Confidential**

**Supervisor's Review of USB RIFCH PhD Thesis**

Surname of the PhD student: <b>Bytyutskyy, MSc.</b>	Name of supervisor: <b>prof. Ing. Martin Flajšhans, Dr.rer agr.</b>
Title of PhD thesis: <b>Interrelationships between ploidy level, genome size and cell size in series of ploidy level models from 2n to 14n fish</b>	

**OVERALL COMMENTARY ON THE PhD THESIS**

Dmytro Bytutskyy entered my lab in 2009 with MSc. in biophysics and his task was to adapt and further develop methods of computer-assisted 2-D and 3-D image analysis to measure DNA content and to assess its nucleotypic effect in fish models of different ploidy levels. Dmytro consequently became familiar with tissue sampling, flow cytometry, microscopic techniques and 2-D imaging, Feulgen image analysis densitometry, confocal laser scanning microscopy for 3-D imaging and object reconstruction. He spent the mandatory 3 months of stay abroad in prestigious laboratories, first in Biological Station of CNRS in Villefranche-sur-Mer (dr. Ch. Rouvier), France where he improved his microscopic and imaging skills, then in the Dept. of Integrative Biology, University of Guelph, Canada (dr. R. Gregory) for comparative DNA content analyses and last in the Russian Federal Research Institute of Fisheries and Oceanography in Moscow (dr. V. Mugue) where he became more familiar with the sturgeon karyotyping issues.

Dmytro Bytutskyy proved his pedagogical abilities as well, he was a consultant of BSc. and MSc. theses of one student, a mentor of summer school students' project and he frequently taught other students and postdocs to use fluorescence- and confocal microscopic imaging, Feulgen image analysis densitometry and flow cytometry. On the other hand, his approach to scientific writing was somewhat hesitating and slower, and as a result he needed to extend his PhD curricula for one extra year in order to complete all duties for PhD thesis defense.

The PhD thesis is conducted as a compilation of three papers published in peer-reviewed IF - journals, with the introductory review on the problems of polyploidy, genome and cell size in Chapter 1. Suitability of tench standard for genome size measurements is highlighted in Chapter 2. Chapter 3 deals with DNA contents from 2n to 12n fish models using Feulgen image analysis densitometry and with assessment of the nucleotypic effect of nuclear DNA content by planar (2-D) imaging, while



Chapter 4 is extended from 2n to recently discovered 14n fish model using also confocal laser scanning microscopy and it evaluates the nucleotypic effect by spatial (3-D) imaging. The following, relatively brief general discussion attempted to summarize the conducted research. However, I would welcome here also more detailed deductions how, knowing the nuclear to cytoplasmic ratio in erythrocytes, the differing shape of erythrocyte nuclei could affect size and shape of entire erythrocytes, how the shifted surface to volume ratio could affect physiological properties of the cells, etc.

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

- 1) Using different fish species and interspecific/intergeneric hybrids from diploids to tetradekaploids, he confirmed that the nucleotypic effect is expressed at each taxonomic level, independently of phylogenetic context and of the ancient- or neopolyploid status.
- 2) He developed a reliable protocol for live cell visualization by confocal laser scanning microscopy, and for 3-D reconstruction of such visualized objects.
- 3) He found that with increasing ploidy level, the nuclear DNA is more densely packed and that for diagnosis of higher ploidy levels, image cytometry is less precise than flow cytometry.

As a supervisor of Dmytro Bytyustkyy, MSc., I can state that the aims of this PhD thesis were fulfilled.

I can see Dmytro as a skilled professional in both planar and spatial image cytometry, as well as in flow cytometry.

Finally, I recommend his PhD thesis for defense.

### **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