



Přírodovědecká  
fakulta  
Faculty  
of Science

Jihočeská univerzita  
v Českých Budějovicích  
University of South Bohemia  
in České Budějovice

## OPPONENT'S REVIEW ON BACHELOR/DIPLOMA\* THESIS

**Name of the student:** Bernhard Mayrhofer

**Thesis title:** Protein Phosphatase 2A in the circadian clock of the linden bug *Pyrrhocoris apterus*

**Supervisor:** Mgr. Vlastimil Smýkal, Ph.D., Mgr. David Doležel, Ph.D.

**Referee:** Adam Bajgar, Ph.D.

**Referee's affiliation:** Department of Molecular biology and Genetics, Faculty of Science

	Point scale <sup>1</sup>	Points
<b>(1) FORMAL REQUIREMENTS</b>		
<b>Extent of the thesis</b> (for bachelor theses min. 18 pages, for masters theses min. 25 pages), <b>balanced length of the thesis parts</b> (recommended length of the theoretical part is max. 1/3 of the total length), <b>logical structure of the thesis</b>	0-3	3
<b>Quality of the theoretical part (review)</b> (number and relevancy of the references, recency of the references)	0-3	3
<b>Accuracy in citing of the references</b> (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
<b>Graphic layout of the text and of the figures/tables</b>	0-3	3
<b>Quality of the annotation</b>	0-3	3
<b>Language and stylistics, complying with the valid terminology</b>	0-3	3
<b>Accuracy and completeness of figures/tables legends</b> (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	3
<b>Formal requirements – points in total</b>		21
<b>(2) PRACTICAL REQUIREMENTS</b>		
<b>Clarity and fulfillment of the aims</b>	0-3	3
<b>Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions</b>	0-3	3
<b>Discussion quality – interpretation of the results and their discussion with the literature</b> (absence of discussion with the literature is not acceptable)	0-3	3
<b>Logic in the course of the experimental work</b>	0-3	3

\* Choose one

<sup>1</sup> Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Practical requirements – points in total		26
<b>POINTS IN TOTAL (MAX/AWARDED)</b>		
	<b>47/48</b>	<b>(0-48)<sup>2</sup></b>

### Comments of the reviewer on the student and the thesis:

The general goal of the thesis was experimentally to determine the role of Protein phosphatase 2A in the regulation of the circadian clock mechanism in *P. apterus*. Based on known transcriptome and sequential homology, seven functional subunits were identified, and their role verified by systemic knockdown. The rhythmicity of locomotor activity and free-running period were used as a phenotypic readout.

The quality of the thesis, simply said, outstanding. The introduction is well-written and logically organized, leading thus reader to clearly defined and reasonable aims of the thesis that can be achieved by proposed techniques. Material and methods represent an excellent part of the thesis, where the author summarized all necessary information for repeating the experiments. Moreover, it makes an impressive list of the techniques that the author has learned during the realization of the project. The results of the thesis are comprehensive and well presented. The gene-specific knockdowns were done in three replications with two dsRNA constructs for each analyzed gene. There are significant differences between the two first trials, probably mainly due to the increased practical experience of the author, and I really appreciate that both these sets were presented separately. The outcome of the thesis is a set of beautiful preliminary data suggesting an important role of the studied gene in the maintenance of circadian rhythmicity and should be addressed into more detail in a follow-up study. The outcomes are discussed and compared with experiments performed previously in *Drosophila*, which makes the body of highly interesting and fluent discussion.

In summary, the thesis clearly documents that student (Bernhard) used offered an opportunity to learn during his bachelor study as much as he can both during his laboratory work as well as during writing of the thesis itself.

Nevertheless, still, there are some questions that can be answered during defense and can serve as a suggestion for writing of the master thesis.

A) The missing part of the project is the quantification of knock-down efficiency when this information is absolutely crucial for the evaluation of the data, can you suggest how to prove the efficiency of the RNAi and in which tissues is knockdown crucial for measured phenotypes.

B) It would also be informative to show the quality of the dsRNA band after reannealing on the gel, to be sure that the injected material is not cleaved by RNases.

<sup>2</sup> Enter the number of points awarded.

C) In part material and methods, there are several methods mentioned without their involvement in the project itself; this seems to be redundant and unnecessary.

D) Could the author suggest how can be the effect of the PP2A knockdown on the running of a circadian clock be established by using methods of molecular biology? Which techniques can be used for the analysis of the direct impact on clock genes cycling and the level of their phosphorylation?

E) Since the PP2A is essential for many biological processes (as it was mentioned in the introduction) could be the decreased motility and vitality in general responsible for observed phenotypes (rhythmicity, change of free-running photoperiod)?

**Suggestions and questions, to which the student has to answer during the defense.**  
**Mistakes, which the students should avoid in the future:**

**Conclusion:**

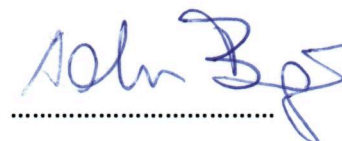
In conclusion, I

**r e c o m m e n d**

**The thesis for the defense and I suggest the grade excellent.<sup>3</sup>**

In České Budějovice

date 23.1.2020



signature

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<sup>3</sup> You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).