STATEMENT OF THE BACHELOR* THESIS REVIEWER

Name of the student: Mykyta Ielanskyi

Thesis title: Analysis of mitochondrial bioenergetics of bloodstream form of *Trypanosoma brucei*

Supervisor: Brian Panicucci

Reviewer: Zdeněk Verner

Reviewer`s affiliation: Dept. Parasitology, Fac. Science, Charles University

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length)**, logical structure of the thesis**	0–3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0–3	1
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0–3	1
Graphic layout of the text and of the figures/tables	0–3	2
Quality of the annotation	0–3	3
Language and stylistics, complying with the valid terminology	0–3	3
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0–3	2
Formal requirements – points in total		15

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*   Choose one

[1]   Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

(2) PRACTICAL REQUIREMENTS

Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	2
Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	2
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	2
Practical requirements – points in total		19

POINTS IN TOTAL (MAX/AWARDED)	48	34[2]

Comments of the reviewer on the student and the thesis:

As with every thesis, upon reading introduction, I was horrified. How could this pass the supervisor's reading? Then, after reading the rest of the work, I calmed down as the rest of the work is well done. Now what is so wrong with the thesis? Citations. Some are outdated; some are missing; and some are simply wrong; moreover, the style changes even for the same article! This is especially striking when going through the list of references at the end of the thesis.

As I mentioned, the rest of the work is more or less well done. I especially appreciate description of the method used as this at least leaves an impression that the student really knows what he has done and why. This part could include composition of the buffers used but then again, the first source in the list of references is "reagent recipes of the laboratory, unpublished". The data are well presented and at the end the student tries to integrate them together and come with a conclusion. The

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[2] Enter the number of points awarded.

discussion itself shows an ability of the author to think about the data and their possible interpretations, however, a discussion with the literature is almost nonexistent. What I really appreciate is the honesty with NMR experiments where the parental strain was somehow swapped. This might have been not mentioned at all and no one would ever know.

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

Mistakes to avoid – style and accuracy of the references. In materials and methods, I miss units used in enzymatic reactions e. g. ligation or restriction. Table 6 lists excessive number of lasers and detectors used for measurement of mitochondrial membrane potential while TMRE is usually measured in PE channel only.

Questions:

1. You mention that there are 24 mitochondrial transporters identified by Colasante et al., 2009. How many of those and how many of novel putative transporters where subsequently identified by MS by Andre Schneider's group?

2. Page 24, last paragraph; also 3.2, page 31: You mention that BF KD of TbAAC shows no growth phenotype and you attribute it to other ways of getting ATP into mitochondrion. As those are unpublished data, could you comment on RNAi efficiency in this strain? Was RNAi efficiency tested by qPCR, Norther or Western blotting?

3. When you tested colonies for insertion of the second part of stem loop vector you used a double digestion of KpnI and BamHI. You concluded that KpnI was a faulty one (page 32, paragraph 4). Why do you think so?

4. When you tested protein levels in DKO A7, you concluded that p18 is reduced (page 35, Figure 13A/B). However, both Fig. 13A shows the same decrease in the second BF lane; at the same time, DKO A7 gives the same signal as parental cell line in the very left lane for both A and B. Did you quantify the signal?

Conclusion:

In conclusion, I

r e c o m m e n d*

the thesis for the defense and I suggest the grade 2 .[3]

In **Prague** date **15/06/18**

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signature

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[3] 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).