Přírodovědecká fakulta
Faculty of Science

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

STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student: Lucia Svoboda

Thesis title: Characterization of mitochondrial proteins in *Trypanosoma brucei*

Supervisor: Priscila Peña-Diaz, PhD.

Reviewer: Ondřej Gahura

Reviewer`s affiliation: Biology Centre CAS, Institute of Parasitology, České Budějovice

	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	2.5
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2.5
Graphic layout of the text and of the figures/tables	0-3	1.5
Quality of the annotation	0-3	1
Language and stylistics, complying with the valid terminology	0-3	2
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	1.5
Formal requirements - points in total		14
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2.5
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality - interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	2.5
Logic in the course of the experimental work	0-3	2.5
Completeness of the description of the used techniques	0-3	1.5
Experimental difficulty of the thesis, independence in experimental work	0-3	3

* Choose one

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

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	1.5
Practical requirements - points in total		20.5
POINTS IN TOTAL (MAX/AWARDED)	**48**	**34.5**

Comments of the reviewer on the student and the thesis:

The overall quality of the thesis is satisfactory, yet with only little bit more effort it could have been much better. The text should have been proofread more carefully, the introduction would strongly benefit from organization into sections/chapters, some data seem to be over-interpreted and/or suggestions for additional experiments are missing. Most importantly, I am missing clearly stated rationale, why the research was performed.

Specific points:

- The title should be more specific (e.g. Characterization of putative constituents of mitochondrial protein quality control machinery in *T. brucei*).
- Some obvious references are missing (e.g. "...70000 new infections in humans..." , "...RNAi was first reported in C. elegans...").
- Figures should be ordered as they appear in the text (see Fig. 3 and 4).
- The link to the TrypTag project website would be useful.
- Figure 3 shows only the N-terminal tagging, while the C-term tagging seems to be more relevant for this work. In addition, the "YFP" label could have been replaced with the "v5" tag, which was actually used.
- Figure 6 is taken from a published article, yet it contains an obvious mistake (which?). The lesson is to check also adapted figures. In addition, the resolution of the (adapted, not original) figure is very poor.
- The second paragraph in 3.1.2 is definitely wrong and incomplete. In general, the methods should be presented so that they are easy to reproduce (ideally without tracing the method several articles to the history). I do not mind if the M&M sections in BSc. theses are written in the concise style as in articles, but then also the quality should be near to the publication level.
- 4.1.: The amount of DNA used is wrong.
- Fig. 7: The expected sizes of fragments would be helpful. Amount of DNA loaded in the lane 1 or 2 is wrong.
- Fig. 8 has an incomplete legend. It is not clear why the figure is shown. The gel is badly overloaded and therefore hardly informative.
- 4.2. requires some conclusion. Which clones are OK? Which were selected for next work?
- Fig 12, upper panel: How do you explain the discrepancy between the strong V5 signal in the WC fraction and very low sum of signal in the remaining fractions? How do you explain the absence of enolase in the WC lane? Given the quality of the westerns, the conclusion in the legend should be more careful.
- 4.3.2.: A proof of downregulation of p4870 is missing. If no proof is available, it should have been stressed. Only in that context the possible (absence of the) growth effect can be discussed.
- Figure 14. Where are the panels A and B? I understand that the letters serve essentially as symbols for this purpose, and therefore one can hypothetically pick any and put them in any

order, nevertheless it is rather unusual not to start with panel A in scientific figures.
- Fig. 17 presents results with a cell line that was not described at all.
- Fig. 16 and 17 were done with different cell lines, therefore it cannot be stated that the results are not reproducible.

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

- What is the similarity of the Clp proteases with the candidate protein(s) on the level of primary and predicted secondary and tertiary structure? Do the candidates bear the features of Clp /AAA+ proteases described in the Introduction? An alignment of amino acid sequences would be informative for the thesis, it could be used also to show the two predicted TMH mentioned in the Results. Also, structures of numerous Clp/Hsp proteins have been determined. A picture of some of them would nicely fit into the Introduction.
- Initially, the Clp proteases are mentioned to localize to mitochondria and chloroplasts in Eukaryotes. But later the text describes cytosolic orthologs. Could you clarify this?
- Could you elaborate on the hypothesis about the loss of Hsp78/104 as a consequence of multicellularity? How does this fit the fact that plants contain Hsp100?
- Why did you choose to study the gene Tb927.11.4870, which, as you stated, "...does not have any well-known homolog..." ? It sounds as if you chose the protein randomly without any further comments.
- Why did you use two different secondary Abs for immunolocalization of p3030. Without explanation it is quite pointless to show both.
- Why do you see enolase sometimes only in the cytosolic fraction and sometimes in both cytosolic and organellar fractions? Does this affect the interpretation of the data (see Fig. 15)? The statement that "...the cytosolic marker enolase were found EXCLUSIVELY in their respective compartments..." is quite wrong in the context of the figure.

Conclusion:

In conclusion, I

r e c o m m e n d

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

In **Stockholm** date **14.6.2018**

signature

[2] 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).