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: Helmut Stanzl

Thesis title: Localization and functional characterization of a mealybug rRNA methyltransferase of bacterial origin

Supervisor: Zdeněk Paris

Referee: Miroslav Oborník

Referee`s affiliation: Institute of Parasitology BC, 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	3
Graphic layout of the text and of the figures/tables	0-3	2.5
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	3
Formal requirements – points in total		20
(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	3
Logic in the course of the experimental work	0-3	3

* Choose one

[1] 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	3
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

POINTS IN TOTAL (MAX/AWARDED)	48	46

Comments of the reviewer on the student and the thesis:

This thesis investigates HGT in a bacterium-within-bacterium-within-insect symbiotic system. The author proved localization of the rRNA large subunit methyltransferase I (Rlm1) in the mealybug bacterial endosymbiont Tremblaya princeps and showed methylation of cytosine in its SSU rRNA at the position 1942. He also localized both endosymbionts in the mealybug by in situ hybridization with specific probes. Although he was not able to show signals of FISH and IFA of Rlm1 at once, the obtained results are with no doubts sufficient for the master thesis and are highly valuable for the field.

I generally have little reservations about the definition of organelle used in the thesis. Frankly speaking, I do not think the question „endosymbiont or organelle" can (and should) be objectively solved. I prefer the view of „endosymbiont or organelles" by John McCutcheon: "In my view it is a continuum, and the definition matters so little, organelles are just very old endosymbionts, that's how I think about it. What you label them is up to you." (Williams 2019, The Scientist). So, for instance, for myself, the symbiont loses its bacterial identity when its genome is lost.

I would be personally careful defining organelle by 10 years old paper, particularly in this highly dynamic scientific field. Any feature stated to be specific for organelles (genetic integration, metabolic integration, and even the host coordinated reproduction) can be found in some typical endosymbiont. So, if the author accepts the cited definitions of organelles, he should state the bacterial symbionts in mealybugs as organelles, and not claim that the results just „blurs the line between endosymbionts and organelles". If he does not believe those definitions, it should be apparent from the thesis more clearly.

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

Page 3:" The genome was found to be missing essential translation-related genes, such as aminoacyl-tRNA synthetases."
Question: Does it mean that there are no aaRSs? Please, explain...

Page 5: „HGT is possible between any two DNA-based life forms."
Question: What other types of life forms do you know? Is it possible to acquire genes from them by HGT?

Page 5: „Little is known about the importance of HGTs in the evolution of classical organelles." I have to disagree; endosymbiotic HGT is crucial for establishing an organelle.

Question: What types of HGT do you know?

Page 6: „In bacteria, the 23S rRNA forms together with the 5S rRNA the 50S rRNA..."
I have serious doubts about this formulation. 50S is a sedimentation rate of all the large ribosomal subunit, that means 5S rRNA, 23S rRNA and usually 34 ribosomal proteins. The two rRNA cannot give together all the mass of a ribosome since rRNA is responsible for about 60% of the ribosome mass (sedimentation rate about 30S).
Question: Can you please describe a structure of a prokaryotic ribosome in detail?

Page 7: „the prediction of targeting signals achieved a high score for some HTGs."
Question: Can you please explain how can you predict HGT by the presence of signal peptide?

Figure 4: Question: Do you have any explanation for the differential expression of exons 1, 2, and 3 in the rlml gene?

Conclusion:

In conclusion, I am happy to r e c o m m e n d the thesis for the defense and I suggest the grade excellent (1) .[2]

In **České Budějovice** date **19. 1. 2020**

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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).