



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: Angela Kienberger

Thesis title: Detection and isolation of relapsing fever spirochete, *Borrelia miyamotoi*, from *Ixodes ricinus* collected in the Czech Republic: separation of relapsing fever and Lyme disease spirochetes by cultivation on solid medium

Supervisor: MSc. Natalie (Natasha) Rudenko, Ph.D.

Referee: doc. RNDr. Alena Žáková, Ph.D.

Referee's affiliation:

	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	3
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
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		19
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
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

* Choose one

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

Logic in the course of the experimental work	0-3	3
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		27
POINTS IN TOTAL (MAX/AWARDED)		48 (46)²

Comments of the reviewer on the student and the thesis:

My comment:

1. I'm missing some photos of *B. myiamotoi* from microscopy, there is only one.
2. The description of Fig. 8 is unclear. The first 2 positive lines are N3-49 isolates and the next 2 positive lines are N3-60 isolates? Positive and negative control are changed in the description. The first is negative, the other is about positive controls.

"Figure 8: Results of nested-PCR of individual colonies with glpQ primers: Lane 1-4: isolate (N3-49) and (N3-60); Lanes 5-6: positive and negative controls; Lane 6: 100 bp DNA ladder

Fig. 9 is also not precisely described:

"Figure 9: Results of PCR with glpQ, p66, fla primers: Lane 1-4: isolates (N3-60(8)) and (N3-60(63)), positive and negative controls with primers for flagellin (506 bp long PCR product); Lane 5: 100 bp DNA ladder; Lane 6-9: isolates (N3-60(8)) and (N3-60(63)), positive and negative controls with primers for glpQ (466 bp long PCR product); Lane 10-13: isolates (N3-60(8)) and (N3-60(63)), positive and negative controls with primers for p66 (532 bp long PCR product)."

In Fig. 12. the labels for the positive and negative control are again reversed.

"Figure 12: PCR analysis with glpQ and ospC primers: Lane 1-3: egg sample, positive and negative controls with primers for ospC (617 bp long PCR product); Lane 4-6: egg sample, positive and negative controls with primers for glpQ (466 bp long PCR product)."

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

Can the student create a picture or map of the tick collection site in the Czech Republic and, if she knows the result of positivity for Bbsl and B.m., mark where it is located.

Did the author only get co-infected isolates? How many isolates did she get in total?

² Enter the number of points awarded.

Neither was with only *B. myimatoi*?

There is no information about the collecting the ticks. Just "Ticks were collected from different areas of the Czech Republic by flagging". Can she give me more?

Conclusion: This bachelor's thesis is precise, it surpasses many diploma theses in the amount of methodologies, text and quality. As an opponent working in a similar field of research, I am very pleased and appreciate its quality. I have to highlight that her results seem to be the first in this case. The author writes "The PCR with the specific primer sets for *glpQ*, sequencing, and the additional reverse line blotting confirmed the presence of a monoclonal culture of *B. miyamotoi*."

Congratulation!

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade A ³

In Brno date 13.1. 2023

signature

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).