Přírodovědecká fakulta
Faculty of Science
Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

STATEMENT OF THE DIPLOMA THESIS REVIEWER

Name of the student: **Alejandro Cabezas-Cruz, DVM**

Thesis title: Cellular and molecular characterization of *Ehrlichia mineirensis* (UFMG-EV), a new organism isolated from *Rhipicephalus (Boophilus) microplus* ticks.
Supervisor: **Prof. Libor Grubhoffer, CSc.**
Prof. Lygia Maria Frishe Passos, PhD.

Reviewer: MVDr. Markéta Derdáková, PhD.
Reviewer` affiliation: Institute of Zoology, Slovak Academy of Sciences, Bratislava

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced extents of the thesis divisions** (recommended extent of the theoretical part is max. 1/3 of the total extent), **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
Adequacy and clarity of the results and conclusions	0-3	3
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 even without reading the rest of the text, explanation of the symbols and labeling, indicating the units)	0-3	3
Formal requirements – points in total		23
(2) PRACTICAL REQUIREMENTS		
Clarity of the aims	0-3	3
Fulfillment of the aims	0-3	3
Discussion quality – interpretation of results and their discussion with the literature	0-3	3
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

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

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 filed and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Formal requirements – points in total		27

POINTS IN TOTAL (MAX/AWARDED)	**51**	**0-51[2]**

Suggestions and questions, to which the student has to answer during the defense:

I really like the complex approach of diploma thesis to characterize newly isolated *Ehrlichia mineirensis* which was done using modern techniques of molecular biology, cell biology, microscopy and immunology.

For the taxonomical classification sequencing and phylogenetic analyses five genes were used. Currently, the Multi-locus sequence typing analysis is common approach used to characterize the bacteria. Do you have any information if this approach is used also for *Ehrlichia?*

Originally, *E. mineirensis* isolate was obtained from *R. microphlus* ticks feeding on the cattle in Brasil. My question is if you have information if there were any clinical signs observed in these animals or these animals had antibodies against *Ehrlichia*? Are there any serological studies on seroprevalence against this bacterium done or are planing in the future?

How would you set up the experiment to test the vector competence of *Ehrlichia mineirensis?*

My last suggestion is, that Abbreviations should be mentioned somewhere in the thesis as separate paragraph, since they are used frequently in the text. What UFMG-EV stands for? I did not find it in the text.

Eventual mistakes, which the students should avoid in the future:

I found some formal mistakes and misspellings but very few and these mistakes don't affect the high quality of the thesis.

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Eventual additional comments of the supervisor on the student and the thesis:

Conclusion:

The diploma thesis has a very high quality both scientific and formal. Author uses different up to date (molecular and cellular) approaches to characterize new species of the *Ehrlichia* genus. The quality of the work is documented by two published manuscripts and one paper that is under review. I think that this outcome is far extending the requirements for the diploma thesis.

In conclusion, I r e c o m m e n d the thesis for the defense and I suggest the grade A.

In **Bratislava** date **12.9.2013**

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signature

[2] Enter the number of points awarded.

Review of the master thesis by Alejandro Cabezas-Cruz, DVM entitled "Cellular and molecular characterization of *Ehrlichia mineirenzis* (UFMG-EV), a new organism isolated from *Rhipicephalus* (*Boophilus*) *microplus* ticks"

The thesis is based on three publications, two of them I found in PubMed database, the third was accepted in *Transboundary and Emerging Diseases*. All of them deal with a new species of *Ehrlichia* isolated from *Rhipicephalus microplus* ticks in Brazil called *E. mineirensis.* Just at the beginning of my review I must emphasize that publication background of this master thesis (two papers with IF > 3) is sufficient for good quality Ph.D. thesis.

Alejandro successfully cultured the newly isolated *Ehrlichia* in cell lines from various hard tick species, but failed in a soft tick cell line. Agents *grown* in *Ixodes scapularis*-derived IDE8 cells he used (with help from Maruška Vancová) for electron microscopy analysis. It was shown that ultrastructure of the new *Ehrichia* species resembles that of *E. canis*, *muris* or *chaffeensis*. At this point I must appreciate these results as I experienced problems with propagation of *Anaplasma phagocytophilum* in human HL-60 cells.

The main part of the thesis is devoted to the phylogenetic classification of the new member of the genus *Ehrichia*. Analysis of five genes generally used for this purpose revealed that UFMG-EV isolate is a new *Ehrichia* species closely related to *E. canis*.

In addition, immunodominat B epitopes were predicted on the putative gp 36 protein of *E. mineirenzis*. Antigenic crossreactivity between *E. mineirenzis* and *E. canis* and *A. marginale* was demonstrated using sera from animals infected with the last two.

Structure of the thesis is "classical", it is not the matter of a cumulative paper thesis. Introduction is rather short dealing with *Ehrlichia* pathogens in common, propagation of *Ehrlichiae* in culture, ultrastructure of ehrlichial agents and their molecular taxonomy. In spite of it is based on 32 citations, introduction is much shorter than usually written by students of the Faculty of Science and this is my only criticism of Alejandro´s thesis.

Used methodology comprises maintaining of several tick cell lines, propagation of *E. mineirensis* in these cells, detection of the pathogen by light microscopy. Samples of infected IDE8 cells were processed for transmission electron microscopy. Methods of molecular biology include isolation of genomic DNA, PCR amplification of selected genes, cloning and sequencing of amplified products. Obtained DNA sequences and translated amino acid sequences were analysed using BLAST and CLUSTALW programmes respectively. The phylogenetic analysis was also performed. Gp36 gene of *E. mineirensis* was analysed for the presence of O- and N-glycosylation sites, two tandem repeat regions were found in this glycoprotein. Prediction of B cell epitopes was performed using B cell Epitopes Prediction Tool as well as 3D structure of the glycoprotein with predicted epitopes. Western blotting was used for demonstration of antigenic cross reactivity between *E. mineirensis*, *E. canis* and *A. marginale*. The methodology is scientifically sound and described in a way that allows reproducing the experiments.

Results are clearly presented and demonstrated in 11 figures and 7 tables. Discussion on 5 pages is comprehensive and documents that Alejandro was really involved in the problem. He demonstrates well-informed view on the problems of polyphasic taxonomy of *Ehlichia* organisms. First of all it is necessary to appreciate identification of a new *Ehrlichia* species which is the first *Ehrlichia* detected in cattle tick in Brazil. Successful propagation of *E. mineirensis* in tick cell lines enables its mass production for further studies. Based on 5 different genes, Alejandro clearly determined phylogenetic position of *E. mineirensis* among other *Ehrlichia* species. Further studies are necessary to analyse the pathogenicity of this agent as well as its ecology including additional vectors and hosts.

Thesis is well written in good English (if I can judge) and brings significant new knowledge in a scientific area.

At the end of the review it behoves to have some questions or comments.

On page 18 you write that phagolysosomes and secondary lysosomes were present in infected IDE8 cells. What is the difference between phagolysosome and secondary lysosome?

In the Discussion you haven´t mentioned the new *Ehrlichia* isolate from jaguars in Pantanal, Brazil (*Ehrlichia* sp. strain Jaguar). Is there any similarity (identity) between this jaguar isolate and your *E. mineirensis*?

MSc

Conclusion: The thesis meets requirements for Mgr. thesis and provides valuable information about a new *Ehrlichia* species. I strongly recommend accepting the candidate's thesis as the basis for obtaining the degree MSc Mgr.

In České Budějovice 12. 9. 2013

Prof. RNDr. Jan Kopecký, CSc.